2019

Implication Of Inorganic Nitrogen And Phosphorous Species As A Cause Of A Harmful Algal Bloom Event In Caesar Creek Lake, Ohio And Its Tributaries

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IMPLICATION OF INORGANIC NITROGEN AND PHOSPHOROUS SPECIES AS A CAUSE OF A HARMFUL ALGAL BLOOM EVENT IN CAESAR CREEK LAKE, OHIO AND ITS TRIBUTARIES

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

By

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B.S., Wright State University, 2019

2019

Wright State University

WRIGHT STATE UNIVERSITY

GRADUATE SCHOOL

December 12th, 2019

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Baxter Jeffrey Foskuhl ENTITLED Implication Of Inorganic Nitrogen And Phosphorous Species As A Cause Of A Harmful Algal Bloom Event In Caesar Creek Lake, Ohio And Its Tributaries BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

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Implication of Inorganic Nitrogen And Phosphorous Species As A Cause Of A Harmful Algal Bloom Event In Caesar Creek Lake, Ohio And Its Tributaries

Agricultural runoff poses a threat to the Wilmington, Ohio municipal water supply and its source waters. A harmful algal bloom (HAB) was documented in June 2019 in Caesar Creek Lake, Waynesville, Ohio. This study seeks to establish baseline nutrient concentrations in Caesar Creek Lake (Warren County, Ohio) and its tributaries, and to identify potential non-point sources of excess nitrogen and phosphorous that contributed to the HAB event. In collaboration with the Wilmington Water Department, dissolved inorganic nitrogen DIN-[N] (sum of NH_3-[N], NH_4^+-[N], NO_2^--[N], NO_3^--[N] concentrations), dissolved phosphorous DP-[P], particulate phosphorous PP-[P], total phosphorous TP-[P] (total sum of DP-[P] and PP-[P] concentrations) and general water quality parameters were measured from May 30th, 2018 to the September 26th, 2019 in Caesar Creek Lake. Dissolved phosphorous DP-[P], particulate phosphorous PP-[P] and total phosphorous TP-[P] (total sum of DP-[P] and PP-[P] concentrations) concentrations were measured on January 23rd, 2019, and from April 9th, 2019 to September 26th, 2019. General water quality parameters, DP-[P] and DIN-[N] samples were taken at depths of 0, -2, -6, and -11 meters. Microcystin samples were obtained and analyzed concurrently by the Wilmington Water Department (WWD). Ion chromatography (IC) was used to determine nitrite, nitrate, and phosphate in

water samples. Sediment samples were obtained from tributaries to Caesar Creek Lake and were analyzed for both TP-[P], DP-[P], and PP-[P] using inductively coupled plasma-optical emission spectrometry at 213.6 nm following acid digestion. A *YSI Professional Plus* electrochemical probe was used to measure general water quality parameters (dissolved oxygen, pH, temperature, specific conductance). The tributaries Turkey Run, Anderson Fork, and Buck Run had the highest measured inputs of DIN-[N] during the 2019 HAB event, with DIN-[N] concentrations of 6.58 mg/L, 3.74 mg/L, and 3.35 mg/L on June 5th, 2019. High precipitation and flow rates into each tributary prevents a definitive claim about a specific tributary potentially causing the 2019 HAB event. There is a clear trend between tributary increases in DIN-[N] and TP-[P] and the increase in microcystin concentrations [MC] in early June of 2019, showing a potential dual role of DIN-[N] and TP-[P] during a known HAB event that occurred from June 1st, 2019 to September 5th, 2019 with spikes of TP-[P] from Turkey Run and Buck Run on June 5th, 2019. Microcystin increased nearly logarithmically during the same time period, and TP-[P] values from Turkey Run and Buck Run found to be 0.318 mg/L, and 0.076 mg/L, respectively. DIN-[N] values from Turkey Run and Anderson Fork, also on June 5th, 2019, were 6.58 mg/L and 3.74 mg/L, respectively. [MC] values increased significantly during the same time period, with values of 0.170 µg/L, 0.365 µg/L, 0.809 µg/L, and 2.608 µg/L on May 30th, 2019, June 6th, 2019, June 13th, 2019, and June 19th, 2019, respectively.

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I. INTRODUCTION

History of Caesar Creek Lake

Caesar Creek Lake is a state park located in Waynesville, Ohio, and is operated by the United States Army Corps of Engineers (USACE).[1, 2] Created in 1978 by the USACE to assist with flood control in the Little Miami River watershed, it now serves as a 19-km^2 (4,700-acre) park and 10-km^2 (2,500-acre) wildlife area.[2] The City of Wilmington, Ohio, uses Caesar Creek Lake as its drinking water source. The Little Miami River watershed consists of small creeks and runs that feed into Caesar Creek Lake, including Anderson Fork, Buck Run, Caesar Creek, Turkey Run, and Trace Run. The land surrounding Caesar Creek Lake is primarily agricultural. The watershed area surrounding Caesar Creek Lake is 627 km^2 (154,880-acre). The following figure 1 is an approximate map of the sub-watersheds and named streams in the City of Wilmington Source Water Protection area.[3] The red star denotes the intake structure at Caesar Creek Lake where sampling occurred, while the white, black, blue, and yellow stars represent the locations of the tributary sites; Caesar Creek, Anderson Fork, Buck Run, and Turkey Run, respectively.

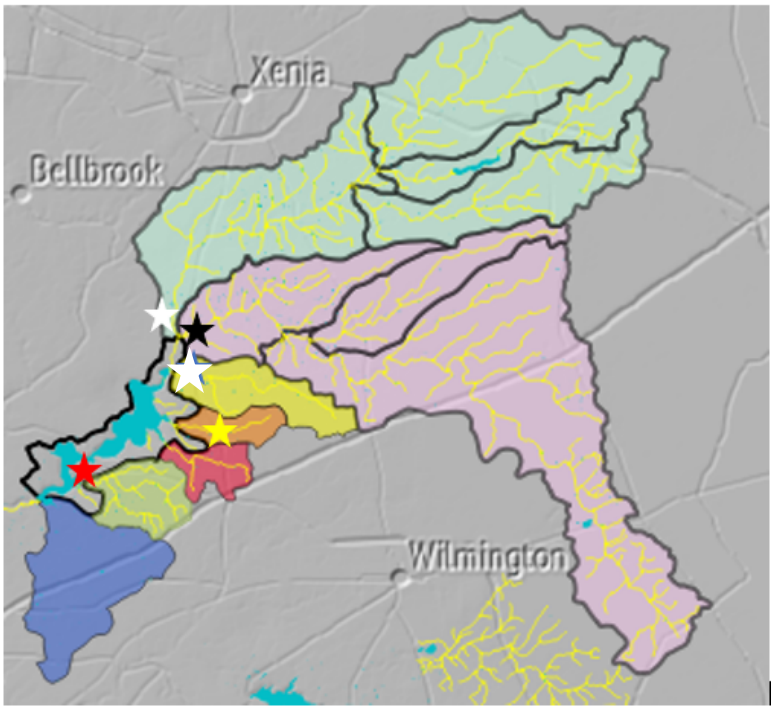


Fig. 1 – Approximate map of the sub-watersheds and named streams in the City of Wilmington Source Water Protection area. The red star denotes the intake structure at Caesar Creek Lake where sampling occurred, while the white, black, blue, and yellow stars represent the locations of the tributary sites; Caesar Creek, Anderson Fork, Buck Run, and Turkey Run, respectively.[3]

History of Harmful Algal Blooms (HABs) in Caesar Creek Lake

For the first time known in Caesar Creek Lake's history, a harmful algal bloom (HAB) was documented in the summer of 2017, yet there was no HAB event during the summer of 2018. Additionally, the second known recording of a HAB was documented on June 1st, 2019. Agricultural runoff is a non-point source of nutrients, such as nitrogen and phosphorous, that algae can feed on to bloom and form HABs. HABs can produce Microcystin-LR, a hazardous cyanotoxin to both children and adults.[36] EPA drinking water health advisory limits are 0.3 µg/L and 1.6 µg/L, respectively.[36] Reported health effects of microcystin exposure include gastroenteritis, liver and kidney damage, allergic reactions, hay fever-like symptoms, and skin rashes.[36] It is possible that potential leaching of contaminated groundwater could cause spikes in dissolved inorganic nitrogen, exacerbating HAB events, or potentially be the cause of HAB events. This is one of several ways nitrogen could affect HAB events. Due to nitrogen- and phosphorous-based fertilizers being used intensively in the watershed, it is clear that

strategies for nitrogen and phosphorous fertilizer runoff control are needed. Both HAB events confirm the need for continued monitoring of Caesar Creek Lake for nutrient concentrations that can lead to these blooms.

The bloom that occurred in Caesar Creek Lake from June to July of 2019 was likely *Aphanizomenon*. *Aphanizomenon* is a filamentous blue green algae that can look like grass clumps. Figure 2 is a slide of a water sample at 400X magnification from June 6th, 2019 from Caesar Creek Lake, Ohio collected by Wilmington Water Department's (WWD) Source Water Protection coordinator, Travis Luncan.[3]:

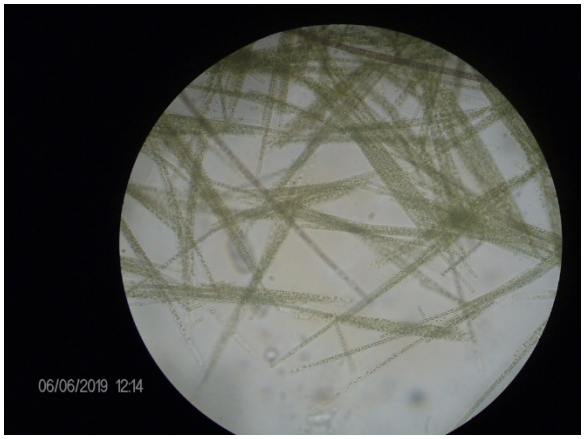


Fig. 2 – A likely *Aphanizomenon* strain of filamentous blue green algae from Caesar Creek Lake, Ohio. The sample was collected by Travis Luncan, Source Water Protection Coordinator of WWD on June 6th, 2019 at 12:14 p.m. at the WWD's intake structure at Caesar Creek Lake, Ohio. The magnification was 400X.[21]

The detection of *Aphanizomenon*, a cyanobacteria that can produce the toxins cylindrospermopsin, anatoxin-a and saxitoxin, was a factor in leading the WWD to switch to their back-up source water reservoirs in June 14th, 2019.[3] Data that warranted the decision to switch to the back-up drinking water reservoir were visual identification of large algal growth in the lake, microscopic identification of *Aphanizomenon*, elevated microcystin concentrations relative to readings normally measured in Caesar Creek Lake as determined by the ELISA method (as part of compliance), phycocyanin readings, microcystin gene abundance by qPCR and satellite imagery of the HAB event.[3]

HAB Chemistry

Enriched systems

A subset of plankton species that are harmful to human health because they produce toxins are called "Harmful Algae".[4] Yet the cause of a HAB event has eluded researchers for decades. Drivers, such as temperature, light, nitrogen, phosphorous, water flow, and precipitation for enriched systems have been well established.[5, 6, 7, 8, 9, 10] The role of nutrient concentrations, however, related to the toxicity, timing, and magnitude of HAB events have so far been ambiguous and hard to track down. Recent literature within the past decade has implied that inorganic nitrogen inputs to well-mixed lakes could control the timing of a HAB event.[6, 11, 12, 13] Enrichment is not absolute, rather, it is a continuum to describe the quality of water both chemically and physically. Enrichment does not necessarily mean that any HAB event will occur, or that it will correlate with time, either. HABs do not occur solely by nutrient loading or mixing. HABs can be controlled by drivers other than nutrients, temperature, and light, such as: flush rate, light shading, seasonal mixing at deep depths in a lake, and herbivory by aquatic organisms.[7] Nutrient requirements can vary significantly for different strains of algae, and different taxa of algae can vary in their ideal TN: TP ratio requirements.[7] There have been some studies that have claimed that the best quantities for describing most nutrient limitations in lakes of any kind were dissolved inorganic nitrogen DIN-[N] to total phosphorous TP-[P] ratios (DIN-[N]: TP-[P]) and DIN-[N] to DP-[P] ratios(DIN-[N]: DP-[P]).[5, 12] It follows then that to begin to determine the role of nutrient concentrations in HAB events, it is necessary to accurately and consistently quantify inorganic and organic nutrient sources before, during, and after HAB events.[14] Figure 3 is a schematic showing the different drivers that could possibly accelerate or trigger cyanobacterial growth (C.B.) that causes HAB events:

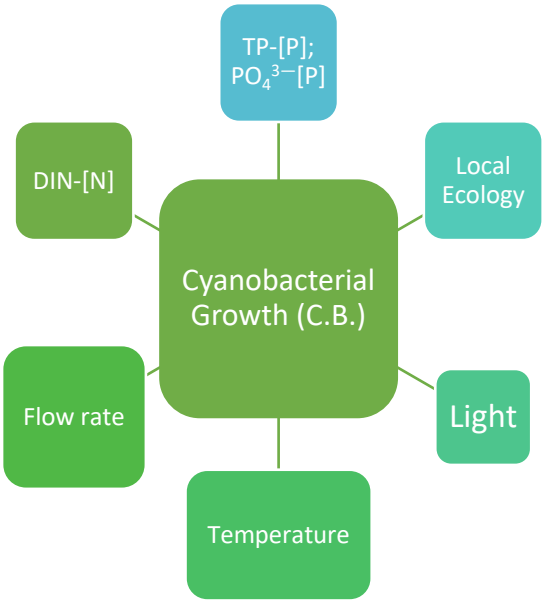


Fig. 3 – Schematic showing drivers that can control HAB events. Local ecology is defined as any ecological interactions that might increase or limit growth of cyanobacteria (C.B.) and its surrounding microorganismal community.[4]

Nutrient Models

It is generally accepted that phosphorous and nitrogen inputs to a body of water of interest, when in favorable temperature conditions, can cause HABs.[4] Some authors have said that the evidence relating to anthropogenic enrichment is ambiguous, unclear, and not enough to correlate the timing and presence to HAB events.[4, 6] Flynn and co-workers attempted to model enrichment's effect on phytoplankton growth by stating that the utilization of nitrogen and phosphorous is determined by the intracellular concentrations of micro-organisms, not the ratios of nitrogen and phosphorous available in the environment.[14] Different models attempt to predict phytoplankton concentrations, such as the Monod "Cell-quota" theory.[14] The Monod theory uses nutrient ratios to determine limitations of nutrient concentrations and how they might affect phytoplankton growth in a system.[14] Some literature has proposed that nutrient ratios would only be important if one or the other, N or P, is far greater than the other. [7, 14] In contrast to the Monod "Cell-quota" theory, Flynn and co-workers suggested that nutrient concentrations measured in summer months rather than N:P ratios are most likely to determine phytoplankton abundance, though they are not the absolute indicator of whether a HAB event would

occur or not.[14] Predictions given by textbooks and review article authors of how nitrogen can be limiting in lake systems are variable, ambiguous, and inconsistent.[7] Inorganic nitrogen species, at low concentrations such as ammonium, nitrate, and nitrite are likely to limit phytoplankton growth, but phosphorous can be limiting in some circumstances as well.[7, 14]

Role of micronutrients

Other nutrients, such as Si, Fe and C, play an important, but much less significant role in the accelerated growth of phytoplankton communities, and potential HAB formation.[14] Carbon deficient systems are known to slow the growth of phytoplankton only under extreme low inorganic carbon concentrations.[7] Si is generally known as less of an anthropogenic influence, but some literature has demonstrated that the N:Si ratio can promote dinoflagellate dominated communities in some systems.[14]

Experimental Lakes Area (ELA) and original phosphorous model

A seminal study was done by David Schindler at the Experimental Lakes Area (ELA) in Canada. Schindler concluded that phosphorous inputs were important, but he did not neglect nitrogen deficiency and the additive effects of combining phosphorous and nitrogen inputs.[7] From both a regulatory and a policy perspective, it is less expensive to establish a cap on the usage of phosphorous-based fertilizers than to attempt to regulate and or limit nitrogen-based fertilizer usage. Additionally, according to Schindler's theory, phosphorous limitation can be anthropogenically induced even if the system is nitrogen controlled.[7] In other words, according to Schindler's model nitrogen deficient systems might exist, but practically it would be easier to limit phosphorous.[7] Historically this theory has prevailed because of its implications on phosphorous management. The consequence of assuming phosphorous always being a limiting nutrient is that in all cases the reduction of phosphorous of input will have a reduction on the frequency and magnitude of HAB events.

Nutrient stoichiometry

There have been attempts to link nutrient stoichiometry and nutrient measurements to the timing, toxicity, and magnitude of HAB events. This simplified model of nitrogen and phosphorous nutrient

dynamics is generally known to be poorly understood and without a real theoretical basis.[12] The reason that the correlation between nutrient stoichiometry and HAB events is so poor is that the time and space variability of nutrients and organisms is incredibly complex, and cannot be possibly modeled in a such a simplified manner.[7] A key flaw with the idea of nutrient stoichiometry involves the uncertainty surrounding the availability of "assimilable", or organism-available N and P fractions in lake water.[7] There is even contention about what is considered "assimilable", since nitrate, for example, can be used by microorganisms if under severe nutrient deficiency (instead of NH_4^+).[11, 14] To add to the complexity, the fact that certain N_2 (an atmospheric form of nitrogen) fixing genera and non-N_2 fixing genera can compete in the same system means different genera can have different nutrient quotas. Even with nitrate being water-soluble in lakes, it is important to note the amount of a nutrient, even if water soluble, does not necessarily imply its availability for phytoplankton uptake and its chemical transformation to a different species.[7]

It is generally not feasible to duplicate field results in a laboratory setting, since laboratory studies are incredibly simplistic relative to field studies, and controlled conditions do not take into account the nutrient loading and the complex nature of drivers of algal growth.[14] Laboratory populations differ from field populations in their nutrient requirements, and thus rates of nutrient uptake in field samples are difficult to accurately compare to laboratory samples.[7]

Practical aspects of nutrient enrichment and regulation

A new, proposed model for nutrient enrichment by researchers such as Schindler and Gobler is one where organismal growth, specifically phytoplankton, is limited in natural lakes by anthropogenic N:P inputs and ratios.[6, 7] Even so, the essential idea behind the control of over-enrichment in individual lakes remains the same as it was under the Schindler (ELA) phosphorous theory of nutrient control: the most promising management and regulatory choice for the control of phytoplankton growth in most situations is the restriction of phosphorous supply.[7] This is not to say that phosphorous limitation alone

will prevent HABs. In some situations, however, in phosphorous enriched systems, reducing phosphorous inputs from anthropogenic sources does not make a difference in inducing a limitation for algal nutrients. The background phosphorous concentrations in either water or soil might be capable of satisfying the total phosphorous nutrient requirement for organisms in a N-limited system.[7] Due to either phosphorus or nitrogen being higher in some systems rather than others, nutrient control and land management should be evaluated on a system by system basis, individual to each lake, or any source water where HAB events might threaten the quality of the drinking water.[7, 14] Some systems might need anthropogenic nitrogen limitation, and some might need anthropogenic phosphorous limitation. Some might need both forms of limitation. As a result of the uncertainty of how and when a lake might be nutrient-limited, nutrient draw-down and recycling makes it difficult for researchers to determine whether or not blooms are a cause of consequence of nutrient scarcity or nutrient loading.[15]

Role of phosphorous

Phosphorous primarily exists chemically as orthophosphate (PO_4^{3-}), a part of organic phosphorous in DNA and RNA, polyphosphates, and inorganic phosphate compounds, which can either be dissolved or adsorbed onto particulate matter in a water body. Structures of possible compounds are shown in Appendix C. Phosphine, the known gaseous form of phosphorus, was invented as a chemical weapon. There is no atmospheric removal mechanism for phosphorous as there is for nitrogen so phosphorus tends to accumulate and recycle in a lake, particularly in lakes with low flush rates.[16] In aquatic systems phosphorous concentrations are far less in the water column than they are in sediments due to low solubility products. For example, with a solubility product (K_{sp}) of $10^{-60.5}$, hydroxyapatite is a phosphate mineral ($Ca_5(PO_4)_3(OH)$) found in sediments that will hardly be soluble in aquatic systems.[17] Phosphorous, then, primarily exists in aqueous conditions as orthophosphate or as a solid adsorbed onto particulate matter or settled in sediment.[16, 18]

Anthropogenic imbalances of inorganic phosphorous species have caused a shift in the natural global biogeochemical budget.[18] As a consequence of over-industrialization and the frequent use of

intensive agricultural practices involving phosphate-based fertilizers for decades, phosphorus concentrations in soils and aquatic systems overall are estimated to be 75% higher than in pre-industrial times.[18] Total phosphorous percentages in surface soils are exceedingly low relative to other elements found in soils, and have been empirically found to between 0.005% and 0.15% in composition.[18]

In the environmental science literature relating to HABs, and analysis of phosphorous in general, there are field-specific operational definitions of different phosphorous species.[19, 20, 21, 22] Dissolved inorganic phosphorous DP-[P] is defined as what can pass through a 0.20-μm or 0.45-μm filter.[22] In contrast, particulate phosphorous PP-[P] is what cannot pass through a 0.20-μm or 0.45-μm filter.[22]

Role of nitrogen

Nitrogen, in contrast to phosphorous, can be transformed into many different species via the nitrogen cycle.[16, 23] Several pathways change the state of matter of nitrogen, and this further complicates the role of nitrogen as a nutrient.[16, 23] Denitrification and nitrogen fixation both allow for the release of N_2 gas and integration of N_2 gas from the atmosphere, respectively, and some cyanobacteria are N_2-fixing bacteria.[16, 23] In addition to natural transformations of nitrogen, synthetically, the Born-Haber process was designed to produce ammonia for agricultural practices. [16] The Born-Haber process, which describes the synthesis of ammonia, is given as expression (1a):

$$N_2 + 3H_2 -> 2NH_3 \quad (1a)$$

In nature, nitrogen can also be fixed by nitrogenase (generalized as a bacteria or organic matter that catalyzes reactions, $\{CH_2O\}$, in the presence of water and an acid to form ammonium and carbon dioxide gas, as shown in reaction expression (1b):

$$3\{CH_2O\} + 2N_2 + 3H_2O + 4H^+ \rightarrow 3CO_2 + 4NH_4^+ \quad (1b)$$

Ammonium can then be oxidized to nitrite, and subsequently further oxidized to nitrate in expressions (2a) and (2b).[16, 23] This process is called nitrification.[16, 23] Of the following nitrogen cycle

processes, nitrification is the most thermodynamically favorable, with a Gibb's Energy of $10^{7.59}$.[16] These reactions are bacteria-catalyzed, where *Nitrosomonas* brings about the expression (2a), and *Nitrobacter* brings about the oxidation of nitrite:

$$2\,NH_3 + 3O_2 = 2\,NO_2^- + 2\,H^+ + 2\,H_2O \quad (2a)$$

$$2\,NO_2^- + O_2 = 2\,NO_3^- \quad (2b)$$

These reactions simplify to expression (2c):

$$2\,NO_2^- + 2\,NH_3 + 4O_2 = 2\,NO_3^- + 2\,H^+ + 2\,H_2O \quad (2c)$$

Denitrification, or the conversion of nitrogen with an oxidation number change of +5 to 0, is shown by the following pathway in expression (2d). Denitrification needs acid and organic matter ($\{CH_2O\}$) to proceed to evolve nitrogen gas:

$$4NO_3^- + 5\{CH_2O\} + 4H^+ \rightarrow 2N_2 + 5CO_2 + 7H_2O \quad (2d)$$

Nitrate reduction is the process of nitrogen with an oxidation state of +5 being reduced to an oxidation state of +3, facilitated by microorganisms in anoxic environments, shown in reaction expression (2e):

$$2NO_3^- + \{CH_2O\} \rightarrow 2NO_2^- + H_2O + CO_2 \quad (2e)$$

All of these nature-driven processes, except the Born-Haber process, have the possibility to occur in lakes and sediments.[16]

Cyanobacteria measured at Caesar Creek Lake

The result of HAB events can be the production of cyanobacteria, which causes the production of cyanotoxins such as microcystin-LR, saxitoxins and anatoxin-a, which can cause harm in vertebrate organisms and humans at μg/L concentrations.[4]

Principle groups of cyanobacterial toxins and their sources include hepatotoxins, neurotoxins, and several others. Within hepatotoxins there are microcystins, nodularins, and cylindrospermopsins. Different kinds of neurotoxins include anatoxin-a (which includes homoanatoxin-a) and saxitoxins.[4] At Caesar Creek Lake, microcystins, cylindrospermopsins, anatoxin-a, and saxitoxins were only sporadically measured from May to August 2019 during the HAB event. The reason for these sporadic measurements was that the WWD only sampled for these cyanotoxins on a as needed basis, and not for research purposes.[3]

Characterization of HABs

Identification of HABs

HABs can be characterized and measured by satellite scans, as well as chlorophyll and microcystin concentrations. Quantification of algal nutrients is important to characterize and measure the extent of HABs. Particulate and dissolved phosphorous quantities are also important to measure because they can tell how much orthophosphate might be available in the water column or adsorbed on particulate matter in the water or if it might exist as organophosphorus. The United State's EPA test for algal growth potential involves the addition of P, N, and N + P, to filtered lake water using growing cultures of *Selenastrum capiconutum.*[7] The *Selenastrum* bioassay has been evaluated by several authors in the literature, and has been found to not be a reliable indicator of algal growth potentials in field populations.[7]

Characterization of the quality of a body of water, including the measurement of pH, dissolved oxygen (DO), specific conductance, and temperature is important to show the general conditions in which a HAB forms. In addition, DO and temperature measurements can indicate the degree of thermal

stratification in a lake, and also be an indication of the availability of oxygen for the transformation of nitrogen species.

Analytical Methods for phosphorous

Measurement of nutrients can be accomplished by using several different analytical methods. Colorimetry, also known as the "molybdenum blue" method, can be used for analyzing phosphate, and is very selective, and affordable.[24] EPA method 365.1 is the method for colorimetric orthophosphate analysis.[25] Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) can be used for the complementary analysis of dissolved, particulate, and total phosphorous.[19, 22, 26, 27]. Filtering samples before analysis using ICP-AES can allow the analyst to measure DP-[P], PP-[P], and TP-[P] concentrations. SOPs 7.4 and 7.5 (see Appendix B) were used for the filtration and acid digestion of PP-[P] and DP-[P], respectively. ICP-AES may be useful if there is a significantly enriched phosphorous environment, or a particularly turbid water body, where PP-[P] could more readily be obtained. ICP-AES is also useful for its ease of operation and wide dynamic range. SOP 5.7 (see Appendix B) was used for the operation and analysis of phosphorous using ICP-AES.

Analytical Methods for nitrogen

Different forms of nitrogen can be measured using a variety of different analytical techniques, such as Ion Chromatography (IC), and colorimetry.[28, 29, 30, 31, 32] Nitrite (NO_2^-) and nitrate (NO_3^-) anions can be detected by IC and optical methods, while EPA methods 352.1 and 353.2 can be used to detect nitrate.[31, 32, 33] An in house standard operating procedure, SOP 4.5 (See Appendix B) was used for the detection and quantification of nitrate and nitrite ions. Ammonium ions can be measured using cationic exchange chromatography, ion-selective electrodes, and optical devices.[33, 34]

Analytical Methods for microcystin and precipitation

Microcystin concentrations ([MC]s), if high enough in magnitude, can be detrimental to human and animal health. The United States Environmental Protection Agency does not officially regulate [MC] on a federal level in drinking water, however, it recognizes [MC] on its Contaminant Candidate List (CCL).[2]

The World Health Organization (WHO), however, has established a provisional guideline for microcystin-LR as 1 μg/L.[35] As a result, ongoing monitoring of [MC] results are important to prevent the endangerment of drinking water. In compliance with EPA Method 546, "The Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay", the WWD monitors [MC].[35, 36, 37] Additionally, for the state of Ohio, the recreational water guideline level for an issuance of a public health advisory is 6 μg/L, while the elevated recreational public health advisory level limit is 20 μg/L.[38]

Precipitation values can help estimate the degree of runoff for a given watershed since runoff often contains a significant amount of nutrients. Precipitation values reported were obtained from the USACE from the Caesar Creek Lake station at Caesar Creek Lake, Waynesville, Ohio.[2]

As a result of this study, the WWD and other Wilmington government officials (Public Health, etc.) will have the baseline data necessary to do further nutrient monitoring. It will be necessary that strategies to combat HABs be continually re-evaluated as more is known about the chemistry of Caesar Creek Lake.

This study sought to examine the role of nutrient concentrations in the timing and extent of a HAB in Caesar Creek Lake. By comparing nutrient and water quality trends to toxin data, the purpose of the study was to implicate a certain nutrient profile (e.g. high nitrogen, low phosphorous) as well as potentially identify runoff from one or more tributaries that might have been linked to the HAB event that occurred from June to July of 2019.

II. EXPERIMENTAL METHODS

Data evaluated

There are several assumptions about the chemical matrix incorporated into the sampling methodology. For example, it is assumed that for a given sample volume, V at a given site, x, V_x, that the concentration (c_x) of the analyte of interest will be uniform from sample collection until the time of sample analysis. It is also assumed that at a given site, that unless obtaining sediment samples that the concentration of the sample of water will not vary significantly as a function of location (samples will not vary if collected at the same GPS location, and does not deviate with GPS location). We also assumed that for samples that were to be obtained for DP-[P] and PP-[P] analysis that the ability of phosphate to readily form insoluble species or to adsorb to particulate matter (humic, fulvic acids in water) would prevent the measurements of DP-[P] and PP-[P] changing significantly between the time that the sample was obtained and the time of sample analysis. We did not and could not easily calculate a percent recovery from our filtration technique. For the acid digestion, we assumed all or near all of the particulate phosphorous due to particulate matter on the filter would be completely digested, accurately reporting a PP-[P] value. This may or may not be true for all samples, and this reflects an error in reporting the concentration of PP-[P].

Total dissolved inorganic nitrogen (DIN-[N]) is defined as the sum of individual inorganic nitrogen species, as nitrogen, in milligrams per liter (mg-[N]/L). The expression for the sum of dissolved inorganic nitrogen (DIN-[N]) values is shown in (3):

$$DIN\text{-}[N] = NH_4^+\text{-}[N] + NH_3\text{-}[N] + NO_2^-\text{-}[N] + NO_3^-\text{-}[N] \quad (3)$$

Total phosphorous is defined in this study as the sum of dissolved, particulate, and orthophosphate, as phosphorous atoms, in milligrams P, per liter (mg-[P]/L). The expression for the sum of total phosphorous (TP-[P]) is shown below as expression (4):

$$TP\text{-}[P] = DP\text{-}[P] + PP\text{-}[P] + PO_4^{3-}\text{-}[P] \quad (4)$$

External sources of data used

Microcystin data was obtained from the WWD as an Excel file. The WWD monitors microcystin concentrations in μg/L as part of compliance with the Ohio EPA for protection of drinking water from cyanotoxins. The WWD obtained microcystin samples from the surface of the intake platform that pumps water from the lake to the city 18.30 km (11.37 mi) east, see Fig. 1. The WWD did not routinely measure *Aphanizomenon* concentrations, though *Aphanizomenon* is a cyanobacteria that can produce the toxins cylindrospermopsin, anatoxin-a and saxitoxin.

If available, precipitation values, reported in cm, were obtained from the USACE for Caesar Creek Lake in the form of an Excel spreadsheet and used for data analysis. The USACE monitors precipitation, flow, and other parameters to monitor the safety of the lake.[2]

Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) for the analysis of dissolved DP-[P], particulate PP-[P], and total phosphorous TP-[P]

To obtain the different fractions of phosphorous DP-[P] and PP-[P], 0.15-μm Whatman glass microfiber filters were used to filter water samples obtained from each site. The 0.15-μm Whatman glass microfiber filters were chosen over 11-μm Whatman #1 filters, as shown in a summary of method development done, seen in Fig. 3D, Appendix D. The filters and aluminum pans used were pre-weighed using a Mettler AE 240 Dual Range analytical balance that is accurate to five decimal places. The samples were filtered, dried in an oven for 72 hours, and dried further under vacuum in a desiccator and a post drying weight was obtained. The dried filter and its filtrate were acid digested according to a standard operating procedure (see SOP 7.4, 7.5 in Appendix B), adapted from EPA method 3050B.[39] The samples were then analyzed using a Varian 710-ES ICP-AES, with an SPS 3 Sample Preparation System (autosampler) following SOP 5.7 (see Appendix B). The concentration of phosphorous in the filtrate was the DP-[P] fraction, while the concentration of phosphorous measured on the filter was the PP-[P] fraction. The replicate read time was either 5 seconds or 10 seconds, depending on the number of samples run. The wavelength of phosphorous used was 213.68 nm. The limit of detection for analyzing total

phosphorous by ICP-AES was calculated to be approximately 0.02000 mg/L total phosphorous. The following Table 1 shows the specifications for the analysis of phosphorous using ICP-AES:

Table 1 – Values of power (kW), plasma flow (L/min.), auxillary flow (L/min.), nebulizer pressure (kPa), replicate read time (s), stab time (s), sample uptake (s), rinse time (s), pump rate (rpm), and number of replicates for the analysis of phosphorous using a Varian 710-ES ICP-AES. The replicate read time was sometimes 5 seconds, depending on the analysis.

ICP-AES Parameter	Instrument Settings
Power (kW)	1.20
Plasma flow (L/min.)	15.0
Auxillary flow (L/min.)	1.50
Nebulizer pressure (kPa)	20
Replicate read time (s)	5 or 10
Stab time (s)	15
Sample uptake (s)	30
Rinse time (s)	10
Pump rate (rpm)	15
Number of replicates	3

Intensity results were then exported as an Excel file for further data analysis.

Ion Chromatography for the determination of Nitrite NO_2^--[N], Nitrate NO_3^-[N], and Phosphate PO_4^{3-} [P]

The following analytes were able to be separated and quantified using a Dionex ICS-1600 Ion Chromatograph (IC) system; fluoride (F^-), chloride (Cl^-), nitrite (NO_2^-), bromide (Br^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), and sulfate (SO_4^{2-}). We defined nitrite as the nitrogen concentration due to nitrite (NO_2^-), denoted NO_2^--[N], µg/mL, and similarly for nitrate, denoted NO_3^--[N]. This method was adapted from EPA Method 300.1 into SOP 4.5 (see Appendix C) and ion

chromatography to determine selected anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-}, and SO_4^{2-}) in water samples.[30]

A Dionex AG22 guard column (4 x 50 mm) was used. The guard column substrate was polyvinylbenzyl ammonium cross-linked with divinylbenzene (55%) with a particle size of 110-µm. A Dionex IonPac AS22 anion-exchange column (4 x 250 mm) with a particle diameter of 65-µm was used. The analytical column was also equipped with a polyvinylbenzyl ammonium cross-linked with divinylbenzene (55%) substrate. The functional group on the column was an alkanol quaternary ammonium with ultralow hydrophobicity. The suppressor used was an ASRS®300 4-mm electrolytic anion suppressor. The autosampler used was an AS40 automated sampler with a 25-µL sample loop with a conductivity detector.

Samples were collected in pre-cleaned, 15-mL plastic test tube vials with polypropylene closures and immediately placed in a cooler and cooled to 4 °C immediately before being transported to a refrigerator in the laboratory and kept at -20 °C for up to 3 months before analysis. Upon thawing, samples were placed for analysis in 0.5-mL Thermo-Fisher polyvials and capped with Thermo-Fisher filter caps. ASTM Type I (18 MΩ or greater) water was used for preparing calibration standards and diluting samples, as needed.

The eluent used for anion IC analysis was a solution of 4.5 mM Na_2CO_3/1.4 mM $NaHCO_3$. The eluent was prepared by pipetting 20.00 mL of AS22 eluent concentrate into a clean 2-L volumetric flask and diluting to two liters. Working standards were prepared by mass using a Mettler Toledo PG504-S Delta Range analytical balance and a Dionex Combined Seven Anion Standard 1 stock solution that contained the following: 50 mL of stock (Cat. No. 056933) that contained 20 mg/L F^-, 30 mg/L Cl^-, 100 mg/L NO_2^-, 100 mg/L Br^-, 100 mg/L NO_3^-, 150 mg/L PO_4^{3-}, and 150 mg/L SO_4^{2-}. The quality control standard (QCS) used was a Sigma-Aldrich Multielement Ion Chromatography Anion Standard Solution, certified (89886-50ML-F, PCODE 101883226). The QCS solution was certified to have 10.0 mg/kg ± 0.2 % of F^-, Cl^-, Br^-, NO_3^-, PO_4^{3-}, and SO_4^{2-}.

Operating parameters, such as flow rate (mL/min.), injection volume (µL), column temperature (°C), cell temperature (°C), suppressor current (mA), and the elution order for the retention times of the analytes are given in Table 2. The following Table 2 shows settings for the parameters for IC operation:

Table 2: IC parameters and their corresponding settings utilized for IC analysis. Flow rate (mL/min.), injection volume (µL), column temperature (°C), cell temperature (°C), suppressor current (mA), and the elution order of the analytes are shown.

IC Parameter	Instrument settings
Flow Rate (mL/min)	1.2
Injection volume (µL)	25
Column Temperature (°C)	30
Cell Temperature (°C)	35
Suppressor current, mA	31
Elution order	F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-}, SO_4^{2-}

Anion identification was based on the comparison of analyte signal peak retention times relative to those of known standards. Quantitation was accomplished by measuring the peak area and comparing it to a calibration curve established from known standards. An example of a chromatogram is given in Fig. 1D, Appendix D.

The Method Detection Limit (MDL) for anion analysis was determined by analyzing the reagent water blank that has been fortified to a concentration that is three to five times the estimated detection limit. The next to the lowest standard solution was used to determine the MDL. Seven aliquots of this solution were analyzed and multiplied by the student's t value, 3.14, for seven replicates and a 99% confidence interval. The MDL was calculated for each individual anion, and equation (3) was followed:

$$MDL = (t) \times (S) \quad (3)$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom, and S = standard deviation of the replicate analyses. The limits of detection of analyzing nitrate, nitrite, and phosphate by IC were calculated to be 0.010, 0.005, and 0.059 mg/L, respectively.

YSI Professional Plus Electrochemical Water Quality Probe for the determination of Ammonia NH_3-[N], Ammonium NH_4^+-[N], and other water quality parameters

The purpose of water quality data was to establish important baseline chemical conditions that could affect nitrogen and phosphorous results obtained, such as dissolved oxygen, pH, and temperature.

Unless otherwise stated, all YSI water quality measurements were accurate to 0.01 units for pH, DO (mg/L), NH_4^+-[N] (mg/L), and NH_3-[N] (mg/L), and 0.1 units for specific conductance and temperature (°C). Limits of detection for NH_4^+-[N] (mg/L) and NH_3-[N] (mg/L) were 0.01 mg/L for both analytes.

The YSI instrument was calibrated before each sampling event (at least 12 hours before, no more than 24 hours before) by placing each calibration parameter in a beaker with a stir bar and stirring for at least 10 minutes. The YSI instrument was calibrated according to SOP 13.0, shown in Appendix B. Data was then stored in as an Excel file and in a notebook for further keeping.

To collect data for a sample, the YSI instrument was placed into the sample, and was set to log data for approximately two minutes. Examples of these plots are shown in Fig. 2D, Appendix D.

Sampling Plan

Tributary sites were selected based on proximity away from Caesar Creek Lake, and ease and safety of access. Lake samples were taken at the intake platform near Harveysburg, Ohio. Travel to the intake site was by a City of Wilmington-owned boat. At Caesar Creek Lake, samples were obtained using a Van Dorn water sampler. In the tributaries, grab samples were obtained about 1 m into each tributary. Samples were collected nearly bi-weekly for 1 year and 4 months, starting on May 30th, 2018, and ending on

September 26th, 2019 through the algae seasons. The following Table 3 shows the GPS location and a qualitative description for each site sampled throughout the project:

Table 3 –GPS coordinates (latitude, longitude), depth of site (m), if applicable, and qualitative descriptions of each site sampled during the project.[20]

Site	GPS coordinates	Lake/tributary site	Depth (m) (if applicable)	Qualitative description
Caesar Creek Lake	39.506719, -84.010558	Lake	0	Grey intake platform with space for sampling, used for control of flow from source water to water treatment plant
Caesar Creek Lake	39.506719, -84.010558	Lake	-2	
Caesar Creek Lake	39.506719, -84.010558	Lake	-6	
Caesar Creek Lake	39.506719, -84.010558	Lake	-11	
Anderson Fork (AF)	39.5681, -83.9649	Tributary	n.a.	Sampled from shore
Buck Run (BR)	39.5466, -83.9708	Tributary	n.a.	
Caesar Creek (CAC)	39.5708, -83.9738	Tributary	n.a.	
Turkey Run (TR)	39.5208, -83.9641	Tributary	n.a.	

The following table 4 is a reference table for the sample days and their corresponding dates:

Table 4 – Sample day table with corresponding dates.

Sample Day #	Date (MM/DD/YYYY)	Sample Day #	Date (MM/DD/YYYY)	Sample Day #	Date (MM/DD/YYYY)
1	05/30/2018	**11**	01/23/2019	**21**	08/13/2019
2	06/13/2018	**12**	04/09/2019	**22**	09/13/2019
3	07/11/2018	**13**	04/23/2019	**23**	09/26/2019
4	07/25/2018	**14**	05/07/2019		
5	07/27/2018	**15**	05/22/2019		
6	08/08/2018	**16**	06/05/2019		
7	09/19/2018	**17**	06/19/2019		
8	10/03/2018	**18**	07/02/2019		
9	10/17/2018	**19**	07/16/2019		
10	10/31/2018	**20**	07/30/2019		

III. RESULTS

PO_4^{3-}-[P] concentrations were measured in Year 1 and 2, but concentrations were all less than the detection limit, or below the laboratory reagent blank concentrations (LRBs) (see Table 1A and 2A, Appendix A). Of the drivers listed in the Fig. 3 schematic, temperature, TP-[P], and DIN-[N] were measured. The tributary sites were not sampled on June 19th, 2019 due to unsafe access to the sampling points. Thermocline data was consistent between Year 1 and Year 2, and is shown in Fig. 4D, Appendix D.

Dissolved inorganic nitrogen DIN-[N], mg/L concentrations

The following figure 4 is a plot of year 1 dissolved inorganic nitrogen DIN-[N], mg/L for the Caesar Creek Lake depths 0, -2 m, -6 m, and -11 m:

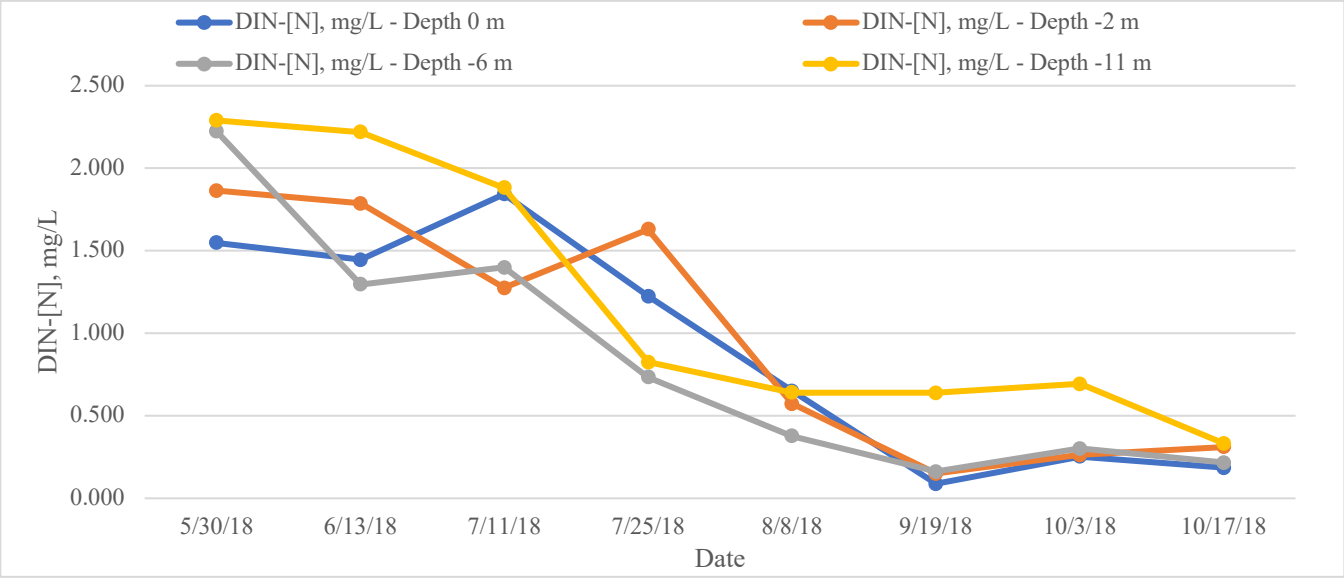


Fig. 4 – Plot of year 1 dissolved inorganic nitrogen DIN-[N], mg/L results for the Caesar Creek Lake depths 0, -2 m, -6 m, and -11 m.

The following figure 5 is a plot of year 2 dissolved inorganic nitrogen DIN-[N], mg/L for the Caesar Creek Lake depths 0, -2 m, -6 m, and -11 m:

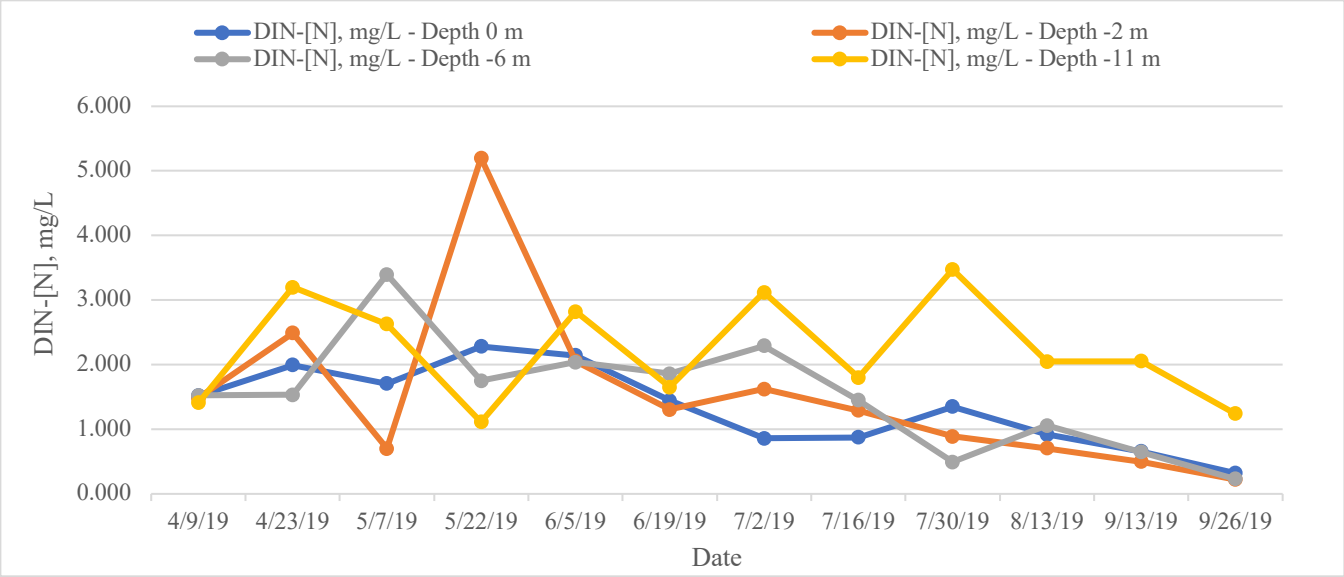


Fig. 5 –Plot of dissolved inorganic nitrogen DIN-[N] for year 2 for the Caesar Creek Lake depths 0, -2 m, -6 m, and -11 m.

The following figure 6 is a plot of year 1 and 2 dissolved inorganic nitrogen DIN-[N], mg/L for the Caesar Creek Lake depths 0, -2 m, -6 m, and -11 m:

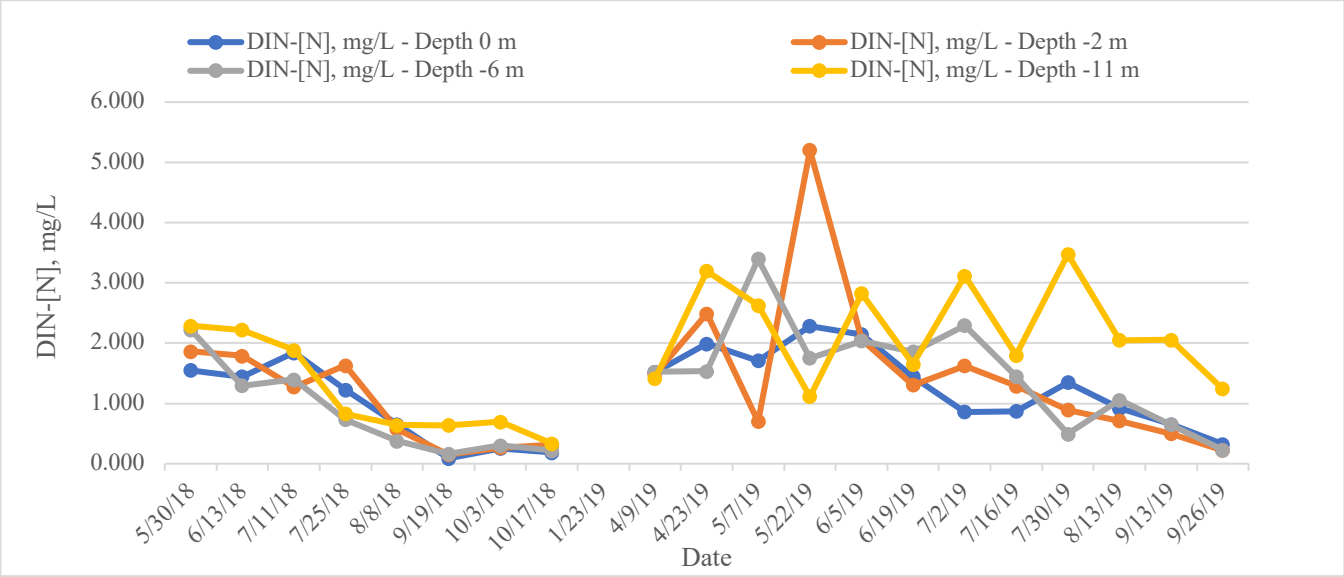


Fig. 6 –Plot of dissolved inorganic nitrogen DIN-[N] for year 1 and 2 for the Caesar Creek Lake depths 0, -2 m, -6 m, and -11 m.

The following figure 7 is a plot of year 2 dissolved inorganic nitrogen DIN-[N], mg/L for the tributary sites Anderson Fork, Buck Run, Caesar Creek, and Turkey Run:

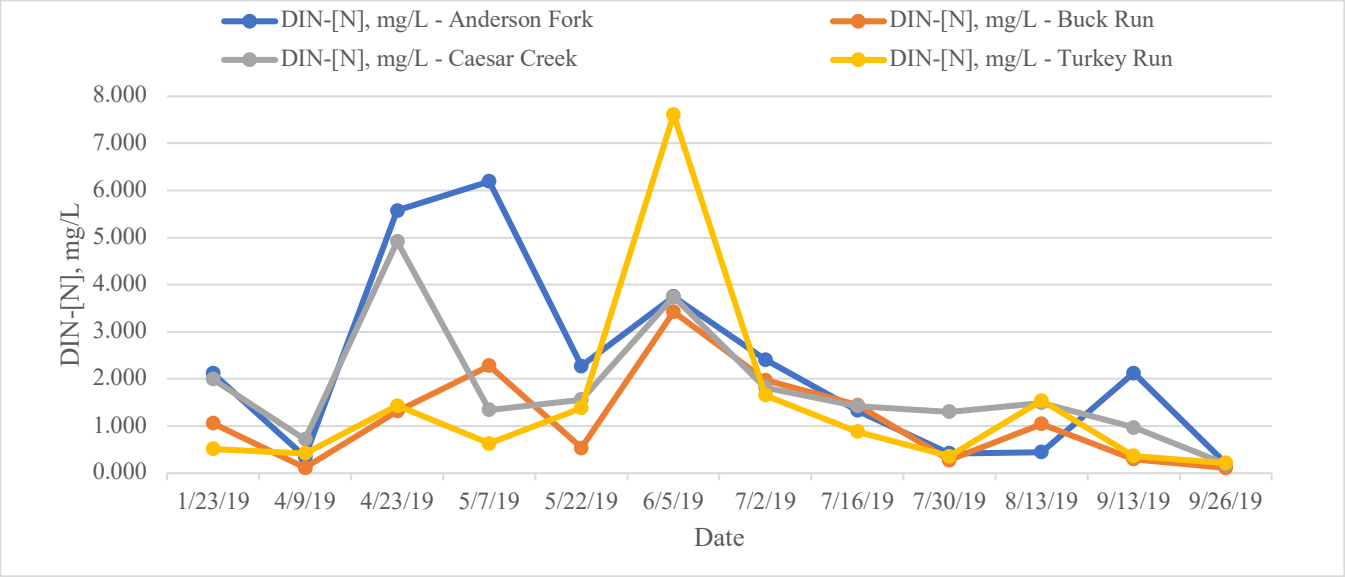


Fig. 7 – Plot of year 2 dissolved inorganic nitrogen DIN-[N] results for the tributary sites

Nitrate NO_3^--[N], mg/L concentrations

The following figure 8 is a plot of year 2 nitrate NO_3^--[N], mg/L for the Caesar Creek Lake depths 0, -2 m, -6 m, and -11 m:

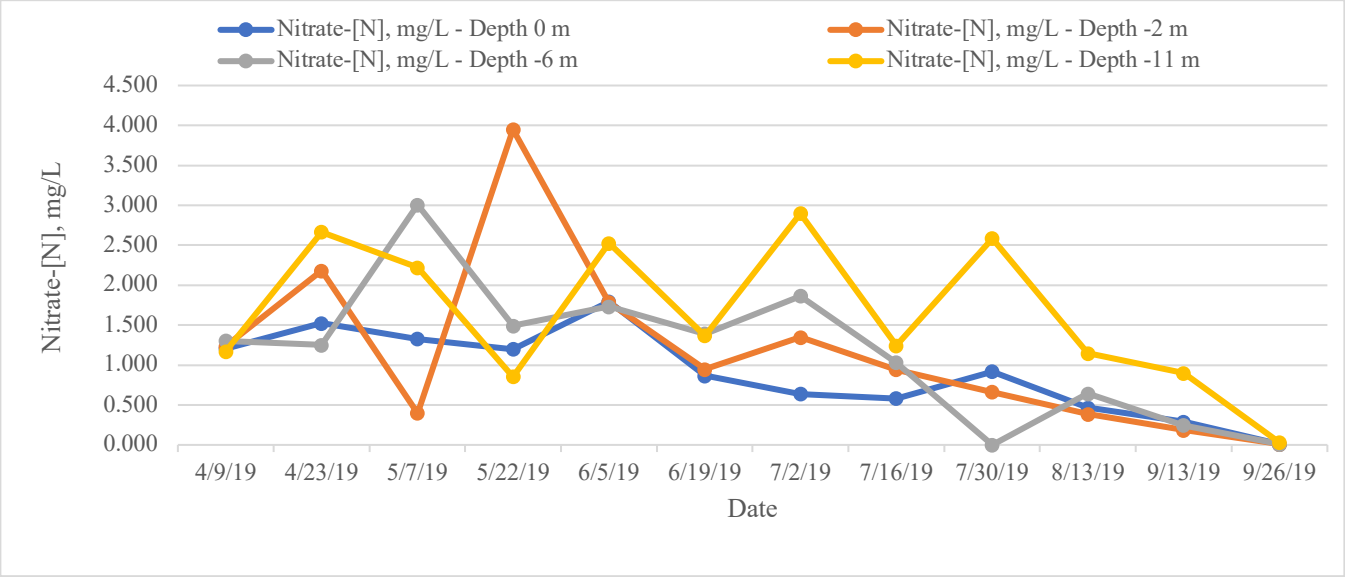


Fig. 8 –Plot of nitrate-N, NO_3^--[N], mg/L for year 2 for the Caesar Creek Lake depths 0, -2 m, -6 m, and -11 m.

The following figure 9 is a plot of year 2 nitrate-N, NO_3^--[N], mg/L for the tributary sites:

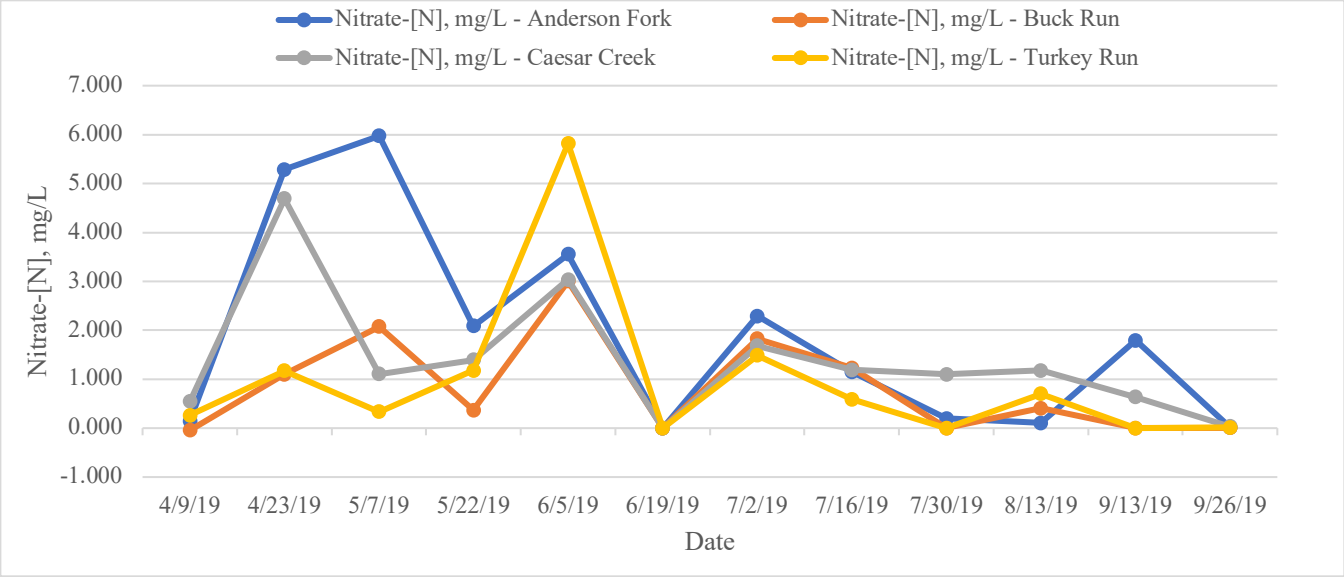


Fig. 9 – Plot of year 2 nitrate-N NO_3^--[N], mg/L for the tributary sites.

Total phosphorous TP-[P], mg/L concentrations

The following figure 10 is a plot of total phosphorous concentrations as TP-[P], mg/L for year 2 for the Caesar Creek Lake depths 0, -2 m, -6 m, and -11 m:

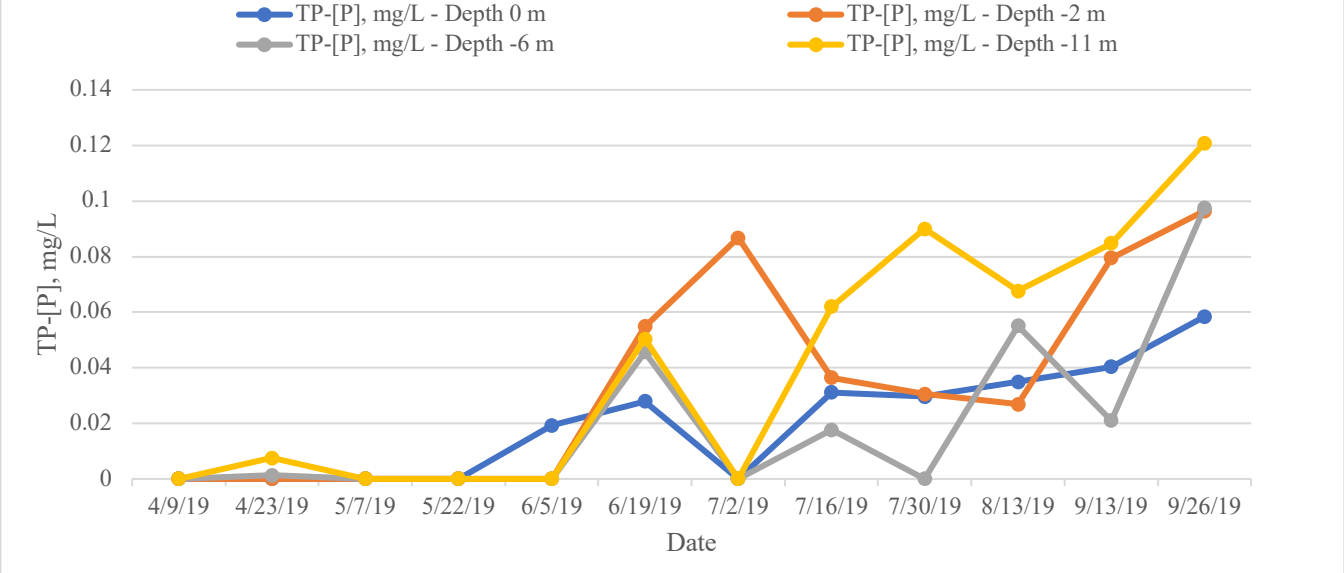


Fig. 10 - Plot of total phosphorous concentrations as TP-[P], mg/L at Caesar Creek Lake for year 2 at depths of 0 m, -2 m, -6 m, and -11 m.

The following figure 11 is a plot of total phosphorous concentrations as TP-[P], mg/L for year 2 for the tributary sites:

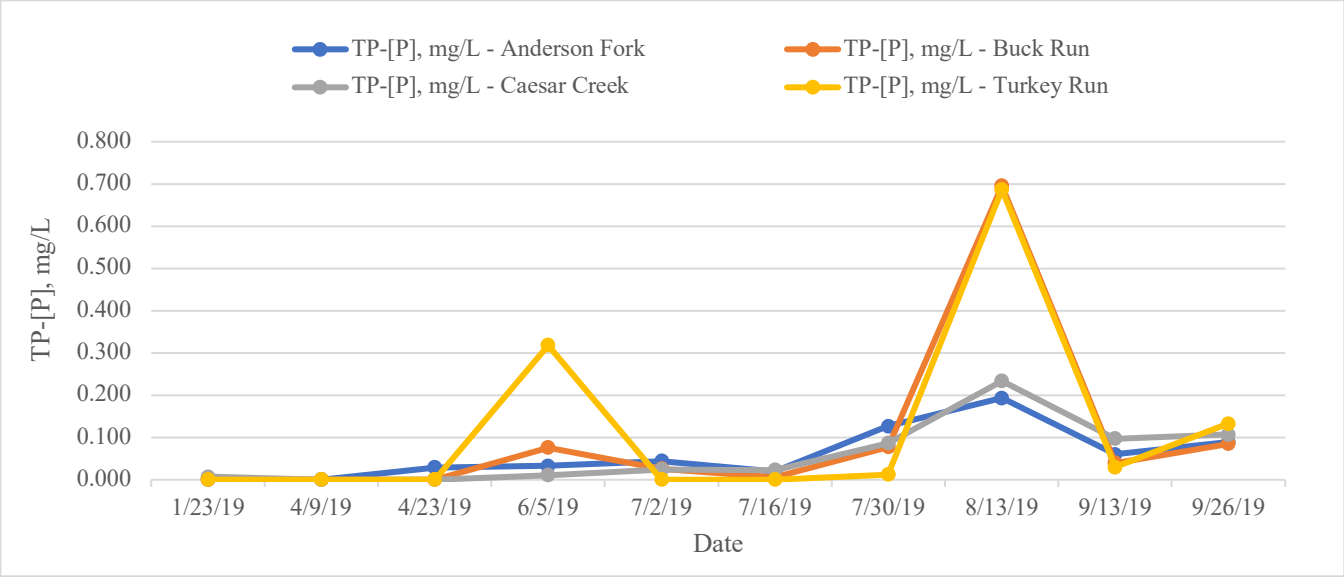


Fig. 11 – Plot of year 2 total phosphorous concentrations as TP-[P], mg/L for the tributary sites.

Ammonium-N, NH_4^+-[N], mg/L concentrations

The following figure 12 is a plot of year 1 and 2 ammonium-N NH_4^+-[N], mg/L for the Caesar Creek Lake depths 0, -2 m, -6 m, and -11 m:

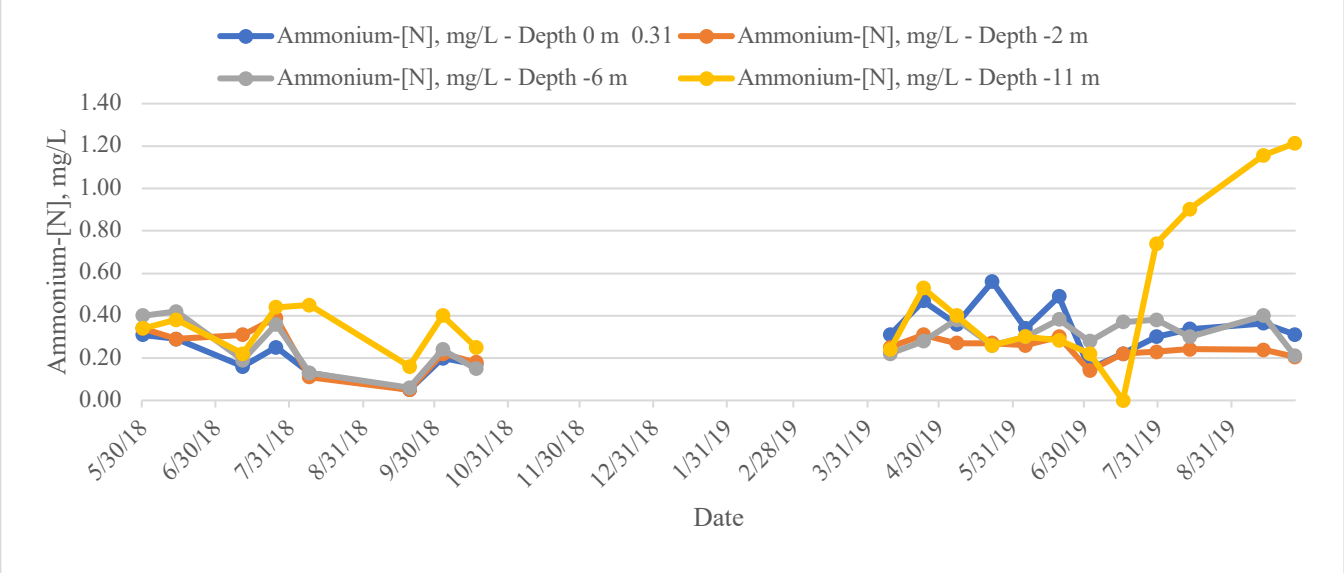


Fig. 12 –Plot of ammonium-N, NH_4^+-[N], mg/L for year 1 and 2 for the Caesar Creek Lake depths 0, -2 m, -6 m, and -11 m.

Molar N:P ratios

Lake - Year 2, Caesar Creek Lake, depths 0, -2 m, -6 m, and -11 m

The following Table 5 are calculated Year 2 molar N:P ratios for the lake depths from April 23rd, 2019 to September 26th, 2019. No DP-[P] or PP-[P] was detected on April 9th, 2019. Some ratios were not able to be calculated due some samples being below the detection limit for phosphorous.

Table 5 - Summary of molar N:P ratios from the lake depths of 0, -2, -6, and -11 meters. No phosphorous was detected on April 9th, 2019.

	Depth - 0 m	Depth - -2 m	Depth - -6 m	Depth - -11 m
4/23/19	n.a.*	n.a.*	516.87	191.33
5/7/19	n.a.*	n.a.*	n.a.*	n.a.*
5/22/19	n.a.*	n.a.*	n.a.*	n.a.*
6/5/19	50.32	n.a.*	n.a.*	n.a.*
6/19/19	23.38	10.79	18.42	14.82
7/2/19	n.a.*	8.46	n.a.*	n.a.*
7/16/19	12.69	16.01	37.19	13.15
7/30/19	20.64	13.21	n.a.*	17.45
8/13/19	11.89	11.94	8.69	13.70
9/13/19	7.33	2.82	13.94	10.96
9/26/19	2.48	1.04	1.06	4.65

n.a.* - Samples where the concentration of phosphorous was below the detection limit of 0.02000 mg/L.

Tributaries - Year 2, Anderson Fork, Buck Run, Caesar Creek, and Turkey Run

The following Table 6 are calculated Year 2 molar N:P ratios for the tributary sites from April 23rd, 2019 to September 26th, 2019. No DP-[P] or PP-[P] was detected on April 9th, 2019. Some ratios were not able to be calculated due some samples being below the detection limit for phosphorous. Samples that are denoted by a "n.a.*" are samples where the concentration of phosphorous was below the stated detection limit of 0.02000 mg/L.

Table 6 - Summary of molar N:P ratios from the tributary sites Anderson Fork, Buck Run, Caesar Creek, and Turkey Run. No phosphorous was detected on April 9th, 2019.

	Anderson Fork	Buck Run	Caesar Creek	Turkey Run
4/23/19	87.23	n.a.*	n.a.*	n.a.*
5/7/19	n.a.*	n.a.*	n.a.*	n.a.*
5/22/19	n.a.*	n.a.*	n.a.*	n.a.*
6/5/19	51.85	20.40	153.07	10.82
6/19/19	n.a.*	n.a.*	n.a.*	n.a.*
7/2/19	24.77	34.55	33.56	n.a.*
7/16/19	30.38	99.07	27.69	n.a.*
7/30/19	1.49	1.63	6.80	12.85
8/13/19	1.05	0.68	2.89	1.01
9/13/19	16.09	3.40	4.52	5.56
9/26/19	1.08	0.60	0.83	0.73

n.a.* - Samples where the concentration of phosphorous was below the detection limit of 0.02000 mg/L.

Year 1 and 2 Microcystin ([MC], µg/L) concentrations at various locations in Caesar Creek Lake

The following Fig. 10 is a plot of microcystin concentrations within and from Caesar Creek Lake. The results for microcystin are a mix between raw source water tested at the WWD treatment facility, samples directly obtained from the lake and sites that were initially sampled by the Ohio EPA during the HAB event that occurred in 2017.

Microcystin concentrations were obtained by WWD as part of compliance with the Ohio EPA requirements for drinking water safety.[36] Tabulated [MC] results are shown in Appendix A, Table.

Year 1 and 2 Microcystin, [MC] concentrations at various locations in Caesar Creek

The following figure 13 is a plot of [MC], µg/L for LT2001 (untreated WWD treatment facility water at tap), RS001 (samples directly taken from Caesar Creek Lake), and L-1, L-2, and L-3 sites were used by the Ohio EPA during the 2017 HAB. The L-1, L-2, and L-3 sites had GPS coordinates of (39.486160, -84.059270), (39.506790, -84.010670), and (39.538230, -83.990990) respectively. [MC],

µg/L concentrations are reported as averages of daily values.

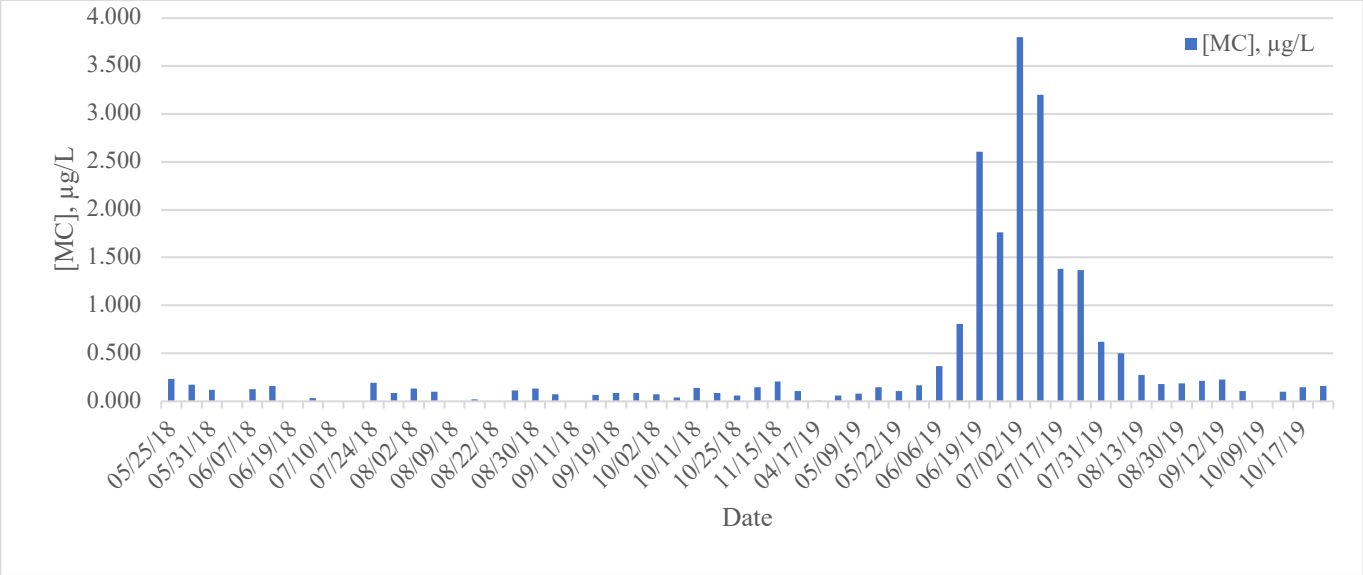


Fig. 13 - Plot of [MC], µg/L concentrations for the LT2001 (untreated WWD treatment facility water at tap), RS001 (samples directly taken from Caesar Creek Lake), and L-1, L-2, and L-3 sites were used by the Ohio EPA during the 2017 HAB. The L-1, L-2, and L-3 sites had GPS coordinates of (39.486160, -84.059270), (39.506790, -84.010670), and (39.538230, -83.990990) respectively. [MC], µg/L concentrations are reported as averages of daily values.[7]

Year 1 and 2 precipitation (cm) values at the USACE monitoring station at Caesar Creek Lake

Lake - Caesar Creek Lake, surface precipitation data

The following Fig. 14 is a plot of precipitation collected by the USACE monitoring at Caesar Creek Lake, Ohio from May 16th, 2018 to October 30th, 2019.

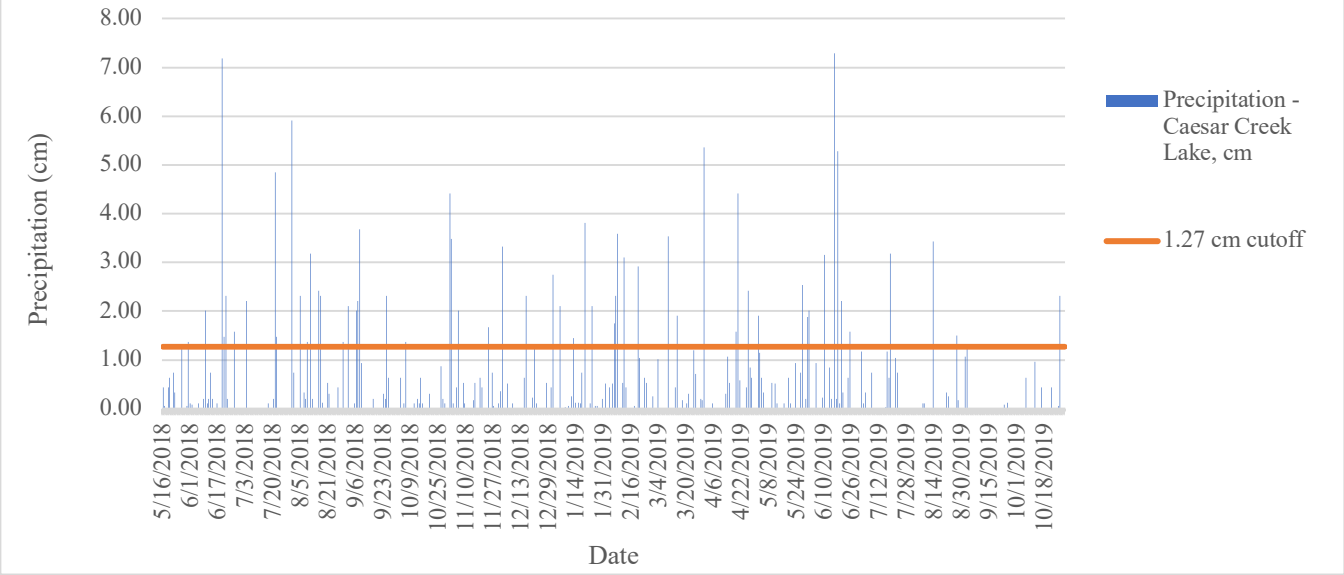


Fig. 14 – Measurements of precipitation (cm) for Caesar Creek Lake, Ohio collected by the USACE from May 16th, 2018 to October 30th, 2019.[37]

IV. DISCUSSION

TP-[P], DIN-[N], and [MC] trends observed

On June 5th, 2019, TP-[P] and DIN-[N] increased at each tributary site. Turkey Run and Buck Run increased in TP-[P] more significantly than Anderson Fork and Caesar Creek, see Fig. 11. [MC] was recorded to be 0.170 µg/L on May 30th, 2019, 0.365 µg/L on June 6th, 2019, and 0.809 µg/L on June 13th, 2019. This near linear increase of [MC] occurred simultaneously with increases in DIN-[N] and in TP-[P]. The increase in DIN-[N] and TP-[P] in the tributaries during that time period is shown in Figs. 7 and 11, respectively. An increase in [MC], DIN-[N], and TP-[P] could be related to a non-point source of nutrient input by one or more of the tributary sites. Additionally, the increase could be replenishment of previously consumed DIN-[N] and TP-[P], and the growth of [MC] was a consequence of low DIN-[N] and TP-[P] measured from April 9th, 2019 to June 5th, 2019. It is possible that Turkey Run, being classified as a run and not a large creek or fork like the other three tributaries, did not have the same magnitude of DIN-[N] but experienced higher degrees of runoff. This trend of DIN-[N] and TP-[P] might implicate Turkey Run as a potential non-point source of excess nitrogen and phosphorous input into Caesar Creek Lake.

Trend of DIN-[N] in Caesar Creek Lake during Year 2

DIN-[N] decreases at all the lake depths except the -11 m site starting from June 5th, 2019 onward for the rest of the sampling season, see Fig. 5. This could possibly mean one of two things. Firstly, it could mean that the inorganic nitrogen was present at -11 m because of the influx of DIN-[N] input from a particular tributary or other non-point source. Secondly, it could mean that the decrease of DIN-[N] was due to the consumption of DIN-[N] by microbial activity that resulted in a diminished nutrient load. The primary amount of DIN-[N] measured was nitrate (Appendix C, Fig. 1C). Since the conversion of ammonium to nitrate is thermodynamically favored and requires DO, non-point source or anthropogenic ammonium concentrations at – 11 m might have been rapidly depleted by microbial activity at a depth of

-11 m. The cyanobacteria present in Caesar Creek Lake during the HAB event could have preferentially consumed ammonium over nitrate, implying that the increase of DIN-[N] at -11 m could have been because of non-point source loading or microbial activity consumption. Complex nutrient cycling systems could change the nitrogen pool profile dramatically in a shorter time span than what we measured.

Presence of NO_3^--[N] at -11 m

Groundwater contamination can originate from agricultural, non-point, septic, and infrastructure sources.[16] Robinson has reviewed the effect of agricultural, non-point, septic, and infrastructure groundwater contamination on the Great Lakes and their tributaries.[40] No studies have been done on agricultural-based groundwater contamination in the watershed of the Great Lake Basin (GLB), however, nitrogen (as N) concentrations were estimated to be 15-70 kg/ha/yr into groundwater in the GLB.[40] Agricultural nitrogen loading estimates are currently unknown for the Caesar Creek Lake watershed, even though agricultural land use predominates. The extensive use of nitrogen-based fertilizers could be a significant source of nitrogen if it contaminates groundwater and leaches into Caesar Creek Lake. Similarly, controlled animal feeding operations, also known as CAFOs might also have an effect on the loading of nitrogen and phosphorus seeping into groundwater that discharges into the Great Lakes.[40] Approximately 4,000 - 6,000 kg/ha/yr. of manure has been produced in southwest Ontario, Canada, whereas in the Caesar Creek Lake watershed, this rate of manure production is currently unknown, and could manure could be a significant source of nitrogen and phosphorous leaching into Caesar Creek Lake from groundwater.[40] There is considerable uncertainty in the volume of groundwater that directly discharges into the Great Lakes Basin, making the level of nutrient pollution into the Great Lakes difficult to determine.[40] Currently, no such estimates of nitrogen or manure are known for Caesar Creek Lake, so the amount of nitrogen contaminating groundwater is unknown.

Nitrate concentrations in Caesar Creek Lake tended to increase below a well-established thermocline in summer months, contrary to expected textbook theory about oxic nitrogen species being present in anoxic waters, seen in Fig. 8.[3] The presence of high nitrate concentrations at depths from -6 m to -11 m

could be explained by the fact that nearby groundwater in Harveysburg, Ohio, is contaminated with excess nitrate ions. Groundwater contamination of nitrate at -11 m could increase the concentration of nitrate measured, skewing the interpretation of the DIN-[N] sum. This is a reasonable suggestion since the sampling intake, measured at GPS coordinates (39.506719 ° N, -84.010558 ° W) is not that far away from the town of Harveysburg, Ohio (GPS coordinates: 39.5037° N, 84.0105° W). The presence of oxidized nitrogen in high concentrations in summer months (specifically April, May, June), might imply that NH_4^+-[N] is consumed in the water column. Additionally, nitrification could be occurring at a rate at which the amount of NH_4^+-[N] is overwhelmingly oxidized to nitrate in anoxic conditions. Low dissolved oxygen concentrations would support this reasoning and are observed at -6 m and -11 meters in Caesar Creek Lake in both Years 1 and 2, see Tables 5A and 6A. Both consumption of NH_4^+-[N] and the oxidation of NH_4^+-[N] to nitrate are plausible explanations for the nitrate concentrations observed -11 m in Caesar Creek Lake. A plot of nitrogen fractions in Caesar Creek Lake for Year 2 is given in Appendix C, Fig. 1C. Groundwater sources of nitrogen and phosphorous, hydrogeological and geochemical processes control the transport of nutrients through the groundwater to Caesar Creek Lake.

Variability of DIN-[N] at -11 m during Year 2

Measurements of [DIN-N] for the -11 m depth of Caesar Creek Lake during Year 2 were more variable than Year 1, as seen in Fig. 6. The correlation coefficient for DIN-[N] values for the Year 1 depth -11 m measurements with respect to the sampling date was calculated to be 0.819. In contrast, the correlation coefficient for DIN-[N] values for the Year 2 depth -11 m measurements with respect to the sampling date was calculated to be 0.356. The difference between these correlation coefficients shows a high degree of variability of DIN-[N] concentrations between Year 1 and Year 2 at the -11 m depth. The variability between Year 1 and Year 2 depth of -11 m DIN-[N] concentrations might indicate a greater degree of microbial activity at -11 m during a season when a HAB event occurred than during a season when a HAB event did not occur. In contrast, the difference between the correlation coefficients could indicate that the DIN-[N] concentrations were normal and had no relation to the occurrence of a HAB

event due to excess DIN-[N], implying a phosphorous limitation in Caesar Creek Lake at that depth. It is possible that *Aphanizomenon*, the harmful cyanobacteria detected during the 2019 HAB event, was controlled by N_2 fixation. The role of *Aphanizomenon* as a N_2-fixing bacteria could mean that cyanobacterial growth in Caesar Creek Lake was not as reliant on the DIN-[N] pool available at the depths measured. Instead, *Aphanizomenon*'s growth could have been controlled by atmospheric N_2 to some degree. If N_2 fixation rates were the predominant nutrient for cyanobacterial growth in Caesar Creek Lake during the HAB event, then DIN-[N] concentrations in Caesar Creek Lake might have been given too much importance in its role of controlling the 2019 HAB event in Caesar Creek Lake.

Timing of increase in DIN-[N] at Turkey Run and increase in [MC]

In early June, the WWD observed that [MC] concentrations were increasing, from 0.170 µg/L on May 30th, 2019, to 0.365 µg/L on June 6th, 2019, and climbing to a concentration of 2.608 µg/L on June 19th, 2019. On June 14th, 2019 the WWD switched their pumps from pumping from Caesar Creek Lake to a back-up water supply. On July 2nd, 2019, the concentration of [MC] peaked, with a concentration of 3.799 µg/L, recorded by the WWD by the ELISA method.[21] On June 5th, 2019, TP-[P] was 0.318 mg/L in Turkey Run, while DIN-[N] was measured to be 6.577 mg/L on that day. Of the four tributaries on June 5th, 2019, Turkey Run had both the highest DIN-[N] and TP-[P] concentrations. June 5th, 2019 can be considered as an inflection point for an increase in [MC], as shown in Fig. 13. On June 5th, 2019, there was a spike in DIN-[N] and TP-[P] from Turkey Run, occurring within the same time period that microcystin concentrations were increasing from June 5th, 2019 to July 2nd, 2019. The spike of DIN-[N] and TP-[P] from Turkey Run could be a result of its proximity to the lake sampling intake structure, see Fig. 1. The timing of this spike from Turkey Run is correlated with the HAB event, but it cannot be determined as the cause of the HAB event due to Anderson Fork having DIN-[N] concentrations of 5.575 mg/L and 6.192 mg/L on April 23rd, 2019, and May 7th, 2019. Both of these dates could imply that Anderson Fork was loading nitrogen into Caesar Creek Lake during this time period leading up to the 2019 HAB event. The spike from Turkey Run could have been correlated with the growth of the

Aphanizomenon and *Microcystin* cyanobacteria if *Aphanizomenon* concentrations were measured during that time period.

Comparison of DIN-[N] to precipitation at tributary sites during Year 2

The DIN-[N] concentrations for all the tributary sites decreased from April 23rd, 2019 to May 7th, 2019, and then sharply increased from May 22nd, 2019 to June 5th, 2019. This decrease in DIN-[N] could be seen as a non-point source of nitrogen input to the tributaries. Additionally, the decrease could be interpreted as a consumption of DIN-[N], followed by either an anthropogenic or non-point source runoff input of DIN-[N]. Precipitation events occurred on April 26th, 2019, May 2nd, 2019, May 28th, 2019, May 31st, 2019, and June 1st, 2019, with 2.41, 1.91, 2.54, 1.88, and 2.01 cm measurements at Caesar Creek Lake, respectively, as shown in Fig. 14.[2] These precipitation events could have diluted the DIN-[N] concentrations by increasing the sample volume. As a result of an increased sample volume, our interpretation of the DIN-[N] increase from April 26th, 2019 to June 1st, 2019 could be an underestimate of the DIN-[N] loading that occurred from April 26th, 2019 to June 1st, 2019.

Comparison of DIN-[N]:TP-[P] ratios to known TN:TP molar ratios

Multiple authors such as Guildford and Hecky and Downing and McCauley have linked high N:P ratios to phosphorous limited phytoplankton growth and low N:P ratios to nitrogen limited phytoplankton growth.[5, 12] By tabulating N:P ratios in hundreds of different lakes and across time Guildford and Hecky conclude that molar TN:TP ratios could predict nutrient limitation in a variety of lakes.[12] Guildford and Hecky reported that nitrogen deficient phytoplankton growth occurred at molar TN:TP ratios of less than 20 (moles total N: moles total P) or 9 by mass (g total N: g total P), and that phosphorous deficient growth occurred when molar TN:TP ratios were greater than 50 (moles total N: moles total P), or 22 by mass (g total N: g total P) in 221 lakes in 14 countries.[12]

Similar TN:TP ratios are reported by Downing and McCauley, who summarized TN:TP ratios found from a variety of different non-point and point sources, such as forests, croplands, and sewage.[5] Downing and McCauley proposed that major sources of TN and TP in a watershed can determine TN:TP ratios

found in lake systems.[5] Drawing from a variety of different nutrient enrichment experiments in current literature, Downing and McCauley proposed that phytoplankton are nitrogen limited when the molar TN:TP ratio is less than 30 in a lake.[5] In this study, we found that our DIN-[N]:TP-[P] ratios were higher in spring months (April, May) than in mid-to late summer months (June, July, August, September) by an order of magnitude. For example, we calculated a DIN-[N]:TP-[P] ratio of 517 at -6 m in Caesar Creek Lake on April 23rd, 2019, whereas September 26th, 2019, we measured a DIN-[N]:TP-[P] ratio of 1.06, as shown in Table 5. A similar trend is seen in the tributary sites, with a DIN-[N]:TP-[P] ratio of 87.2 found for Anderson Fork on April 23rd, 2019, while later in the summer on August 13th, 2019 a DIN-[N]:TP-[P] ratio of 1.05 was measured, as shown in Table 6.

The decrease in DIN-[N]:TP-[P] ratios in Caesar Creek Lake and all of the tributary sites from April 23rd, 2019 to September 26th, 2019 supports Guildford and Hecky's, and Downing and McCauley's hypothesis of total nitrogen to total phosphorous ratios being predicting nitrogen or phosphorous limitation of cyanobacterial growth in a lake system (in this study, Caesar Creek Lake).[5, 12] While Guildford and Hecky and Downing and McCauley measured total nitrogen, our speciation of dissolved inorganic nitrogen is a better estimate of the assimilable, or biotically available nitrogen available to cyanobacteria that could cause HABs in Caesar Creek Lake. While TN:TP ratios might not completely represent the availability of assimilable nitrogen and phosphorous species in a lake or tributary, species ratios can help determine what kind of nutrient limitation exists in the system. For example, NO_3^--[N]:TP-[P] ratios might predict more accurately the biotically available nutrients for cyanobacteria in Caesar Creek Lake if compared to TN:TP ratios that included organic-N. By measuring species-specific ratios, nutrient stoichiometry could be used for the prediction of the type of nutrient limitation and what forms of assimilable nitrogen or phosphorous might exist in a lake or tributary. We can plausibly say that during a bloom year, Caesar Creek Lake is a phosphorous limited system in spring and early summer months, where in later months it is a nitrogen limited system.

Western Lake Erie basin and Lake Mendota

We can compare the nitrate concentrations in Caesar Creek Lake in 2018 and 2019 to those known in the western Lake Erie basin, Ohio from 2012-2014.[6, 41] A total of 19 and 21 sampling events in the western Lake Erie basin, Ohio were done from 2013 and 2014, in addition to samples taken in 2012.[12, 36] They sampled at an average depth of approximately 5 m at four sampling stations weekly, bi-weekly, or monthly from July - September.[6, 41] This is a similar sampling plan that we executed. We sampled 23 times from 2018 to 2019 from eight sites, which included: four different depths at a lake and four different tributaries, see Tables 3 and 4. In addition, Stumpf et al. sampled Maumee River, a significant tributary to Lake Erie, whereas we sampled four different tributaries that feed into Caesar Creek Lake.[6, 41]

The following figure 15 is from a seminal review written by Gobler et al., where nitrate, soluble reactive phosphorous (SRP, µg/L), phycocyanin (µg/L), and particulate microcystin (µg/L) concentrations at approximately 5 m deep in Lake Erie, Ohio, are plotted against Julian day on the x-axis.[6, 41]

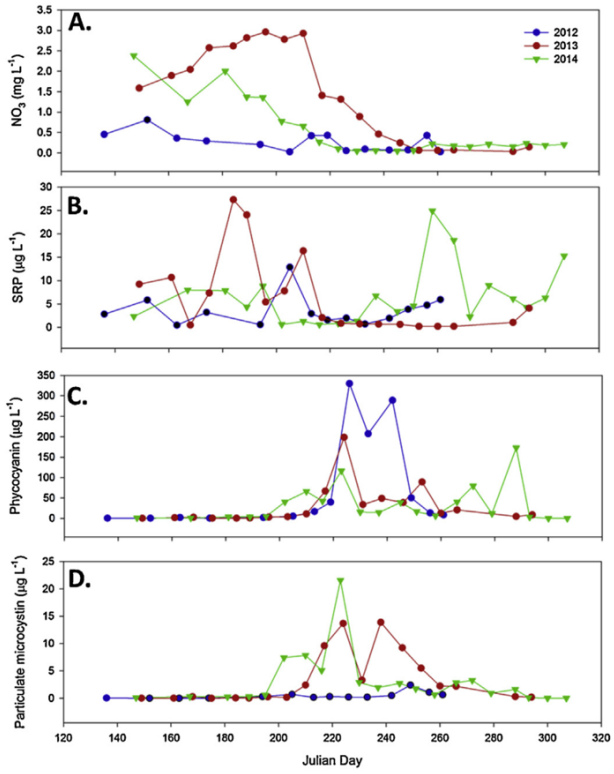


Fig. 15 - Plot of nitrate, soluble reactive phosphorous (SRP, µg/L), phycocyanin (µg/L), and particulate microcystin (µg/L) concentrations with Julian day on the x-axis at approximately -5 m depth in the

western basin of Lake Erie, Ohio for 2012-2014. Used with permission from the journal Harmful Algae, published by Elsevier. [6, 41]

The following figure 16 is a plot of year 1 and year 2 ammonium, NH_4^+-[N], mg/L, nitrate, NO_3^--[N], mg/L, and [MC], µg/L concentrations and year 1 total phosphorous TP-[P], mg/L concentrations at Caesar Creek Lake at - 6 m depth:

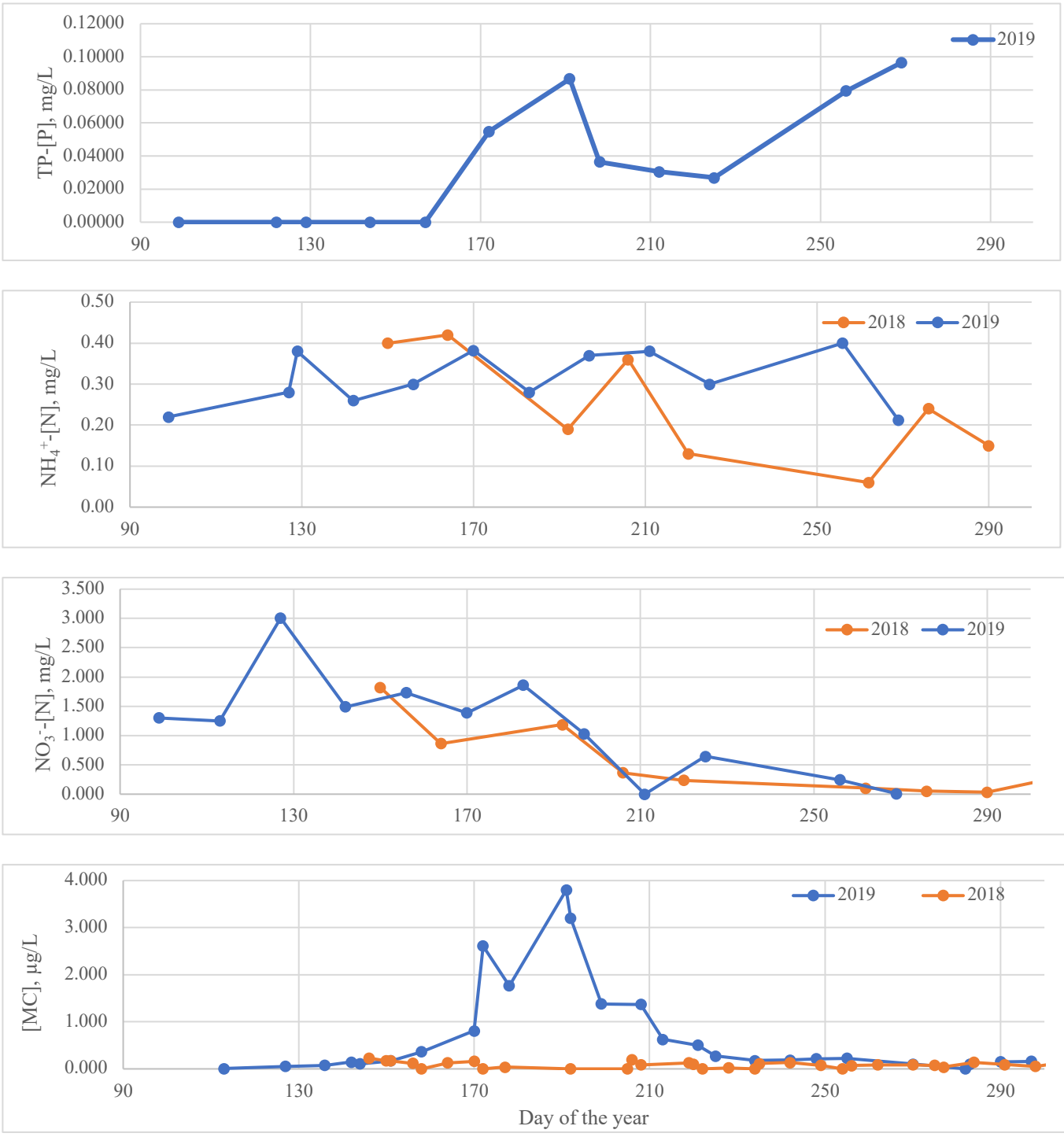


Fig. 16 - Plot of year 1 and year 2 ammonium, NH_4^+-[N], mg/L, nitrate, NO_3^--[N], mg/L, and [MC], µg/L concentrations and year 1 total phosphorous TP-[P], mg/L concentrations at Caesar Creek Lake at - 6 m depth.

Additionally, Fig. 16 has a very similar profile to Fig. 15, inset A.[6, 41] Nitrate concentrations tend to decrease in Lake Erie from about Julian Day 120 to Julian Day 220 in 2012, 2013, and 2014 (see Fig. 15). Similarly, the nitrate concentrations in Caesar Creek Lake at approximately -6 m in depth in 2018 and 2019 display the same decreasing trend from day 125 to 225 (see Fig. 16). In Fig. 15, spikes of increased phycocyanin and particulate microcystin concentrations are seen on day number 220 of the Julian calendar in 2012, 2013, and 2014, while nitrate gradually decreases to a flat line at around Julian day 220 in 2012, 2013, and 2014. Our data suggests that particulate microcystin, like Fig. 15, has a seasonal spike, see Fig. 16. The slow decrease in nitrate as the summer goes on might imply that while the inter-annual variability might be high for the first half of the year, the overall trend of nitrate remains the same for two different systems.

In Fig. 15, SRP reaches a minimum about the same time period particulate microcystin concentrations start to rise. SRP starts to decrease around day 215, while particulate microcystin concentrations peak around day 220. In comparison, we observe a similar trend of [MC] change related to TP-[P] concentrations. In Fig. 16, the peak [MC] concentration was observed on July 2nd, 2019 and occurred on the same day that TP-[P] concentrations were low for the tributary sites. TP-[P] concentrations were 0.044 µg/L, 0.026 µg/L, 0.025 µg/L, and below the detection limit for Anderson Fork, Buck Run, Caesar Creek, and Turkey Run, respectively. The relatively low TP-[P] concentrations for the tributary sites and the spike of [MC] on July 2nd, 2019 are shown in Figs. 11 and 13, respectively.

Maumee River, a tributary of Lake Erie, was sampled from 2012-2014, during the same time period as Lake Erie was being sampled. The following Figure 17 is from a review written by Gobler et al., where Stumpf et al. provide measurements of overlay discharge (ft^3/s), nitrate (mg/L) and SRP (µg/L) from the Maumee River from 2012 to 2014 against Julian day on the x-axis for the years 2012, 2013 and 2014: [6, 41]

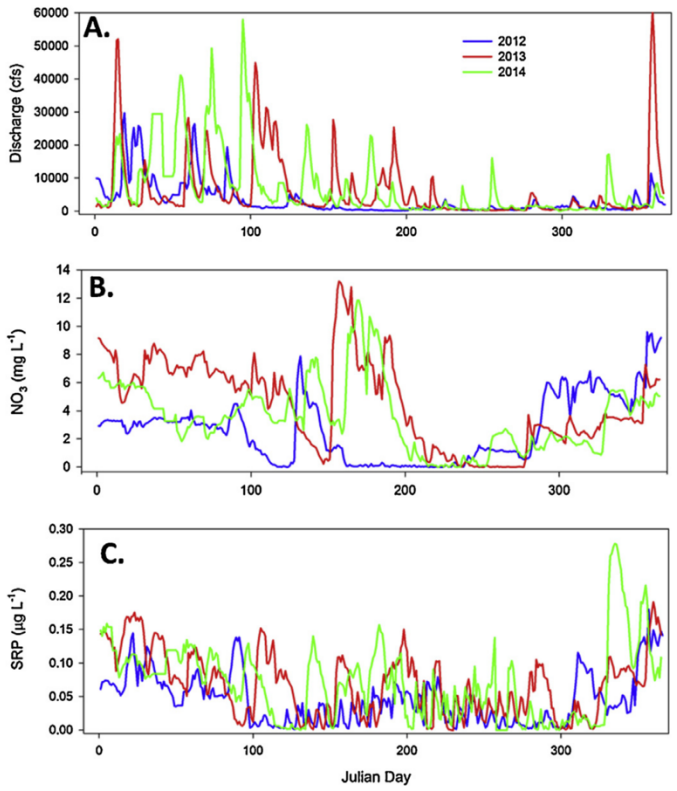
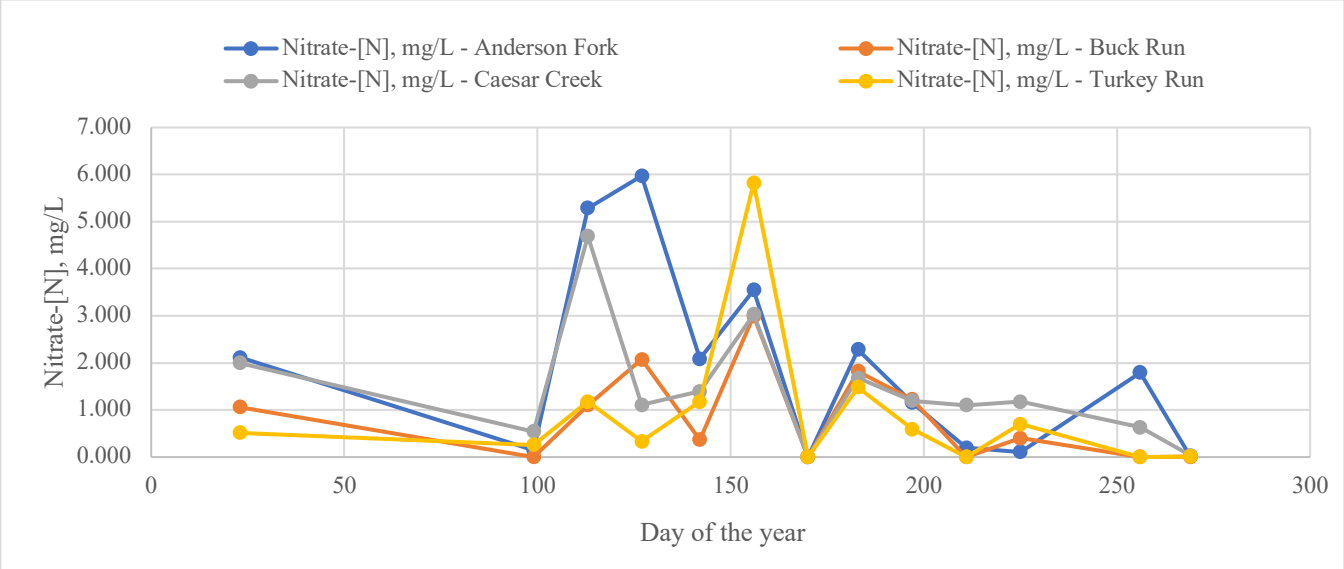


Fig. 17 - Discharge (ft^3/s), nitrate (mg/L) and SRP (µg/L) measurements from the Maumee River, Ohio from 2012-2014 against Julian day on the x-axis. Used with permission from the journal Harmful Algae, published by Elsevier.[6, 41]

The following figure 18 is a plot of year 2 nitrate, NO_3^--[N], mg/L and total phosphorous TP-[P], mg/L concentrations at Anderson Fork, Buck Run, Caesar Creek, and Turkey Run:



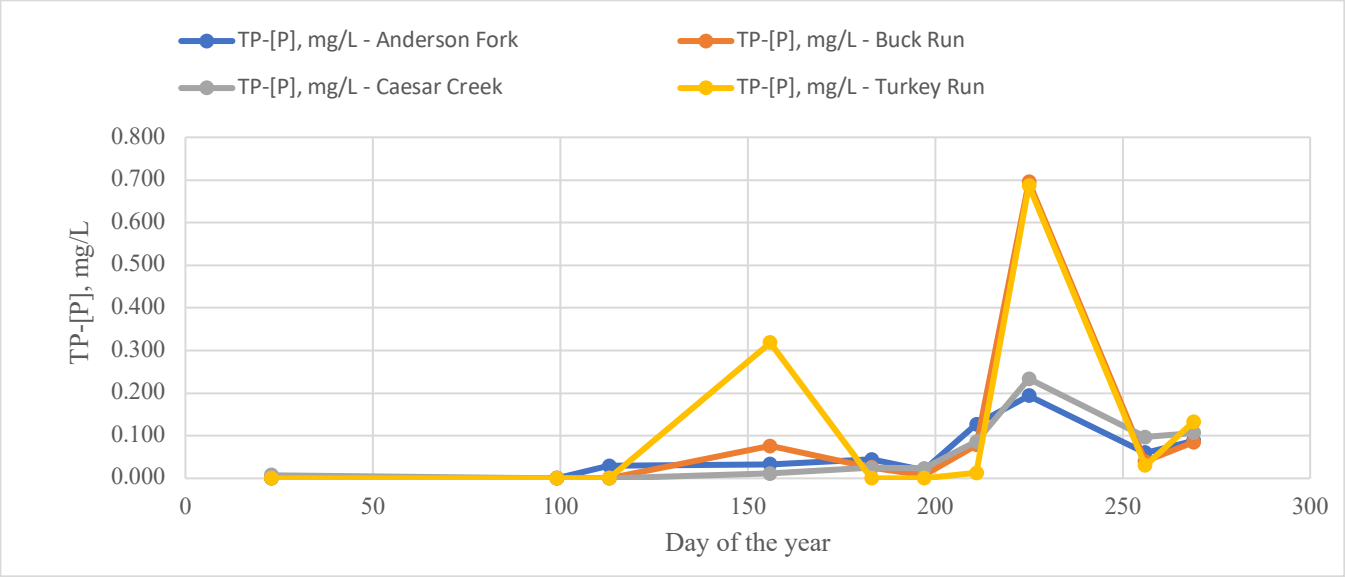


Fig. 18 - Plot of year 2 nitrate, NO_3^--[N], mg/L and total phosphorous TP-[P], mg/L concentrations at Anderson Fork, Buck Run, Caesar Creek, and Turkey Run.

SRP values in the Maumee River in 2013 and 2014 did not show a clear trend (no maxima during the year and between years sampled).[6, 41] In this study, spikes of TP-[P] were seen on days 156 and 225 for all of the tributary sites, with Turkey Run having the highest concentration of TP-[P], mg/L on day 156 and Buck Run having the highest concentration of TP-[P], mg/L on day 225. It is clear that there are significant nitrate concentration spikes were observed in the Maumee River at approximately Julian Day 150 in 2013, and 2014, see Fig. 17. In 2012, there was a significant nitrate concentration spike in the Maumee River at approximately Julian Day 125. In this study, tributary nitrate measurements confirm the findings found by Stumpf et al., as shown in Fig. 8.[6, 41] The peak nitrate concentration this study recorded was on day 127, with a NO_3^--[N] of 5.972 while Stumpf et al. measured a peak of nitrate at approximately Julian day 125 in 2012, which was a non-bloom season.[6, 41] The nitrate concentration spikes in 2013 and 2014 are observed in Lake Erie between Julian Days 150 - 200, while another NO_3^--[N] concentration spike is seen in the tributaries on day 156, with Turkey Run having a peak NO_3^--[N] concentration of 5.817 mg/L. As NO_3^--[N] concentrations decreased from day 127 to 211 (Fig. 16), tributary NO_3^--[N] concentrations, thus, NO_3^--[N] cannot be ignored in establishing the role of nitrogen input on the formation of a HAB event.

A study was done on the role of nitrogen input in the production of *Aphanizomenon* and *Microcystis* cyanobacterial growth in Lake Mendota, Wisconsin.[15] Lake Mendota is a large and temperate natural lake in Wisconsin, United States of America. Beversdorf et al. reported N_2 fixation rates, microcystin, [DIN], [DRP], [TP], microcystin-LR ([MCLR]), relative fluorescence units (RFU), and *Aphanizomenon* and *Microcystis* measurements for Lake Mendota during 2010 and 2011. Their data is summarized in figure 19:

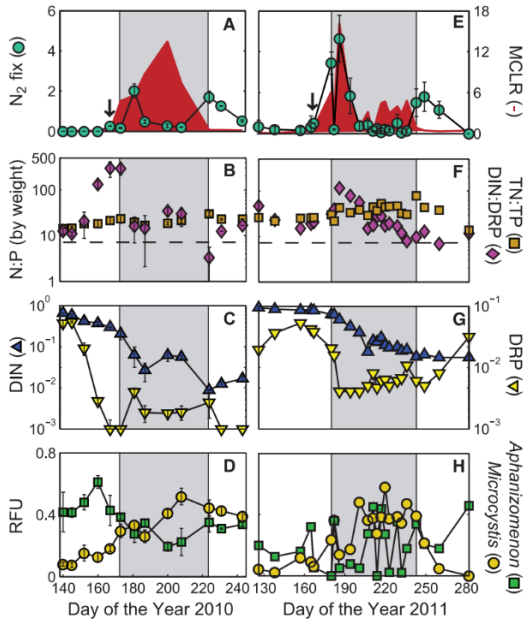


Fig. 19 - N_2 fixation rates, microcystin, [DIN], [DRP], [TP], microcystin-LR ([MCLR]), relative fluorescence units (RFU), and *Aphanizomenon* and *Microcystis* measurements for Lake Mendota, Wisconsin, during 2010 and 2011.[15]

The following figure 20 is a plot of year 2 molar log(DIN-[N]:TP-[P]) ratios for Caesar Creek Lake depths of 0, -2 m, -6 m, and -11 m:

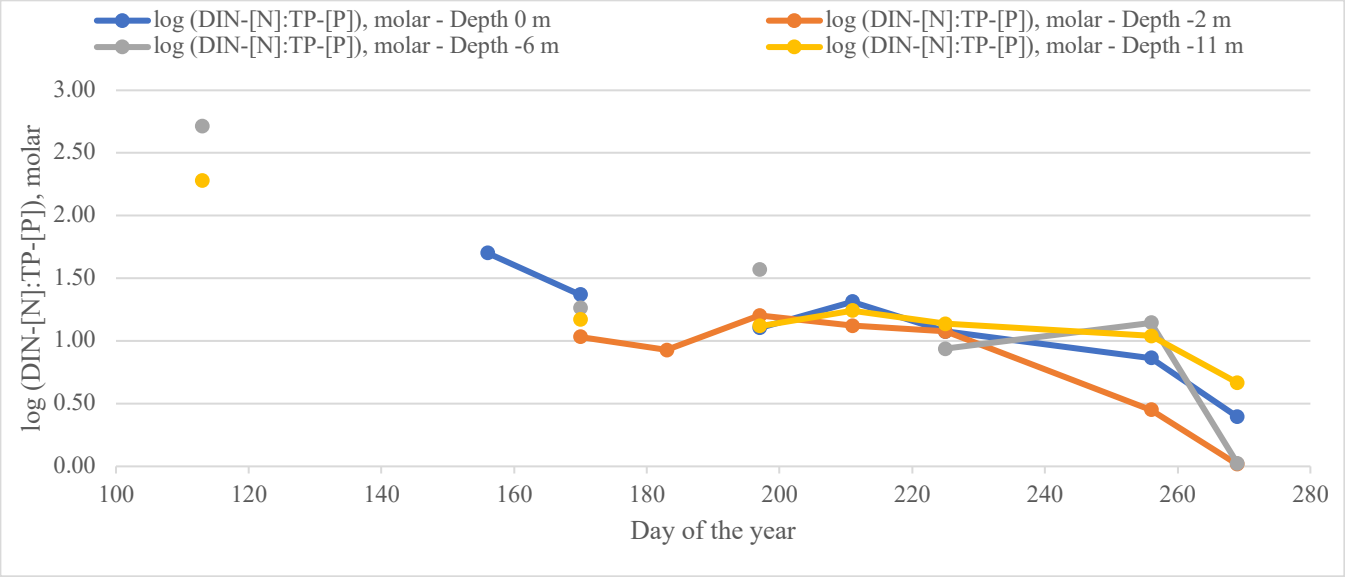


Fig. 20 - Plot of year 2 molar log(DIN-[N]:TP-[P]) ratios for Caesar Creek Lake depths of 0, -2 m, -6 m, and -11 m.

Beversdorf et al. found that following the thermal stratification of Lake Mendota, DIN-[N] concentrations significantly dropped in both 2010 and 2011, which they claim that the cause was large N_2 fixation events in which *Aphanizomenon* mostly participated.[15] Beversdorf et al. claim that the large input of nitrogen, from *Aphanizomenon* led to *Microcystis* blooms that coincided with MC-LR (a particular kind of microcystin) concentration increases.[15] Our data is similar to Lake Mendota, in that we observe N:P ratios that are reported within a similar range. DIN-[N]:TP-[P] ratios by weight were calculated for the tributary sites by multiplying the molar DIN-[N]:TP-[P] ratios by a factor of (14.0067/30.0937), which is the molecular weight of nitrogen divided by the molecular weight of phosphorous. For example, our DIN-[N]:TP-[P] ratios by weight in Caesar Creek Lake ranged from 0.47 (Depth -2 m on September 26th, 2019) to 233.73 (Depth - 6 m, April 23rd, 2019). In the tributary sites, the N:P ratios by weight ranged from 9.52 (Buck Run, August 13th, 2019) to 69.22 (Caesar Creek, June 5th, 2019). A similar significant decrease throughout the summer season in [DIN-N] concentrations are also seen in Caesar Creek Lake during a non-bloom and bloom year, see Fig. 6. While *Aphanizomenon* samples were not obtained as part of routine monitoring like the [MC] samples, a similar trend in [MC] was seen in Lake Mendota in comparison to Caesar Creek Lake. Relatively high N:P ratios by weight, decreasing [DIN-N]

concentrations, and late summer [MC] spikes are observed both in Caesar Creek and Lake Mendota, implying trends seen in two different temperature, potentially eutrophic midwestern lakes.

Consideration of N_2 fixing genera

The role of N_2 fixation was not considered in this study, and reaction (1b) is an important mechanism for the production of ammonium in lake systems. By not measuring the fixation of N_2 to ammonium and the contribution of organic N to the measurement of the total nitrogen pool, a limited nitrogen profile was created. That is not to say that the data measured is inconclusive by any means. By measuring the DIN-[N] and TP-[P] profiles of a previously "unknown" system, the role of DIN-[N] and TP-[P] in the timing of a HAB event can start to be elucidated. Additionally, total phosphorous concentrations are obtained in only the water column (and not the sediment), and the predominant form of phosphorous is orthophosphate, it is reasonable to suggest that whether PP-[P] or DP-[P] is useful information for gauging available P to cyanobacteria in the water column.

V. CONCLUSIONS

The role of declining nitrate concentrations in tandem with low TP-[P] and high [MC] concentrations in late summer months in a bloom season was observed in Caesar Creek Lake and its tributaries, and it is supported by similar trends in Lake Mendota, Wisconsin, and Lake Erie, Ohio.[6, 15, 41] DIN-[N]:TP-[P] ratios were calculated and compared to known values in a variety of lakes and seasons, and our values indicate that cyanobacterial growth in Caesar Creek Lake is phosphorous limiting in spring and early summer (April 23rd, 2019 to June 19th, 2019) and nitrogen limiting in mid-late summer (July 2nd, 2019 to September 26th, 2019). While Turkey Run and Buck Run have the highest measured inputs of DIN-[N], high precipitation and not accounting the flow rate of each tributary prevents a definitive claim about a specific tributary potentially causing the 2019 HAB event. Anderson Fork and Turkey Run are recommended for further monitoring due to Anderson Fork's large size, and Turkey Run's ability to transport large amounts of runoff very quickly into Caesar Creek Lake. Furthermore,

there is a clear trend between tributary and lake increases in DIN-[N] and TP-[P] and the increase in [MC] in early June of 2019, showing a dual role of DIN-[N] and TP-[P] in causing a HAB event.

In a future study, additional methods should be used for analyzing organic nitrogen and orthphosphate. The molydbendum blue spectrophotometric method should be used for analyzing PO_4^{3-}-[P], complementary to the IC phosphate and ICP-AES total phosphorous method used. To analyze organic nitrogen, a method to detect different isotopes of nitrogen using mass spectrometry would have been useful to determine the source and to speciate between the inorganic and organic nitrogen forms in samples. Weekly measurements or remote monitoring set ups would have been ideal to get a more accurate profile of the changes in nutrient concentrations over time. Better estimates of flow rates would help determine the role of runoff. Measuring secchi depth would have helped with understanding effect of light on cyanobacterial growth.

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VII. APPENDIX A – Tabulated results

The results are separated by the instrument or method analyzed to obtain the result. For example, the blue colored tables denotes results obtained by IC. The pink-orange colored tables denote results obtained by ICP-AES, and the green tables denote results obtained by the YSI Water Quality probe.

The following table 1A shows nitrate and phosphate concentrations in mg/L for depths of 0, 2, 6, and 11 m for Caesar Creek Lake for the first year of sampling.

Table 1A – Year 1 nitrate and phosphate results in mg/L for 0, 2, 6, and 11 m at Caesar Creek Lake.

	Nitrate (as NO_3^-, mg/L)			
Sample Date/Depth (m)	0	2	6	11
5/30/2018	5.305	6.572	8.071	8.631
6/13/2018	5.027	6.539	3.834	8.138
7/11/2018	7.316	4.053	5.261	7.357
7/25/2018	4.185	5.311	1.612	1.705
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	2.173	1.915	1.054	0.849
9/19/2018	0.082	0.359	0.455	2.121
10/3/2018	0.144	0.105	0.228	1.252
10/17/2018	0.023	0.385	0.149	0.211
10/31/2018	1.328	1.418	1.115	0.000
	Nitrite (as NO_2^-, mg/L)			
Sample Date/Depth (m)	0	2	6	11
5/30/2018	<LOD	<LOD	<LOD	<LOD
6/13/2018	<LOD	<LOD	<LOD	<LOD
7/11/2018	<LOD	<LOD	<LOD	<LOD
7/25/2018	<LOD	<LOD	<LOD	<LOD
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	<LOD	<LOD	<LOD	<LOD
9/19/2018	<LOD	<LOD	<LOD	<LOD
10/3/2018	< LOD	< LOD	< LOD	< LOD
10/17/2018	< LOD	0.171	0.112	0.127
10/31/2018	< LOD	< LOD	< LOD	< LOD
	Phosphate (as PO_4^{3-}, mg/L)			
Sample Date/Depth (m)	0	2	6	11
5/30/2018	< LOD	< LOD	< LOD	< LOD

6/13/2018	< LOD	< LOD	< LOD	< LOD
7/11/2018	< LOD	< LOD	< LOD	< LOD
7/25/2018	< LOD	< LOD	< LOD	< LOD
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	< LOD	< LOD	0.215	< LOD
9/19/2018	< LOD	< LOD	< LOD	< LOD
10/3/2018	< LOD	< LOD	< LOD	< LOD
10/17/2018	< LOD	< LOD	< LOD	< LOD
10/31/2018	< LOD	< LOD	< LOD	< LOD

The following table 2A shows nitrate and phosphate concentrations in mg/L units for depths of 0, 2, 6, and 11 m for Caesar Creek Lake for the second year of sampling.

Table 2A – Year 2 nitrate and phosphate results in mg/L for 0, 2, 6, and 11 m at Caesar Creek Lake.

	Nitrate (as NO_3^-, mg/L)			
Sample Date/Depth (m)	0	2	6	11
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	5.348	5.472	5.760	5.187
4/23/2019	6.738	9.651	5.543	11.801
5/7/2019	5.877	1.767	13.308	9.827
5/22/2019	5.304	17.482	6.610	3.774
6/5/2019	7.938	7.902	7.662	11.170
6/19/2019	3.831	4.193	6.151	6.059
7/2/2019	2.833	5.959	8.246	12.821
7/16/2019	2.582	4.166	4.564	5.511
7/30/2019	4.070	2.925	n.a.	11.446
8/13/2019	2.056	1.711	2.850	5.081
9/13/2019	1.289	0.828	1.092	3.974
9/26/2019	0.045	0.048	0.043	0.132
	Nitrite (as NO_2^-, mg/L)			
Sample Date/Depth (m)	0	2	6	11
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	< LOD	< LOD	< LOD	< LOD
4/23/2019	< LOD	< LOD	< LOD	< LOD
5/7/2019	< LOD	< LOD	< LOD	< LOD

5/22/2019	2.528	4.837	< LOD	< LOD
6/5/2019	< LOD	< LOD	< LOD	< LOD
6/19/2019	0.435	0.299	0.428	< LOD
7/2/2019	0.195	0.520	0.682	< LOD
7/16/2019	0.240	0.536	0.236	0.275
7/30/2019	0.643	n.a.	0.501	0.725
8/13/2019	0.582	0.395	0.565	< LOD
9/13/2019	< LOD	0.345	< LOD	< LOD
9/26/2019	< LOD	0.027	0.034	< LOD
	Phosphate (as PO_4^{3-}, mg/L)			
Sample Date/Depth (m)	0	2	6	11
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	0.112	< LOD	< LOD	< LOD
4/23/2019	< LOD	< LOD	< LOD	< LOD
5/7/2019	< LOD	< LOD	< LOD	< LOD
5/22/2019	< LOD	< LOD	< LOD	< LOD
6/5/2019	< LOD	< LOD	< LOD	< LOD
6/19/2019	< LOD	< LOD	< LOD	< LOD
7/2/2019	< LOD	< LOD	< LOD	< LOD
7/16/2019	< LOD	< LOD	< LOD	< LOD
7/30/2019	< LOD	< LOD	< LOD	< LOD
8/13/2019	< LOD	< LOD	< LOD	< LOD
9/13/2019	< LOD	< LOD	< LOD	< LOD
9/26/2019	< LOD	< LOD	< LOD	< LOD

The following table 3A shows particulate phosphorous [PP] (mg/L), dissolved phosphorous [DP] (mg/L), total phosphorous [TP] (mg/L), and total phosphorous [TPDW] in mg/kg dry weight for the first year of sampling for Caesar Creek Lake at depths of 0, 2, 6, and 11 meters.

Table 3A – Year 1 fractional phosphorous data sorted by [PP] (mg/L), [DP] (mg/L), [TP] (mg/L), and [TPDW] (mg/kg dry weight) for Caesar Creek Lake at depths of 0, 2, 6, and 11 meters.

	Dissolved P [DP], mg/L			
Sample Date/Depth (m)	0	2	6	11

5/30/2018	n.a.	n.a.	n.a.	n.a.
6/13/2018	n.a.	n.a.	n.a.	n.a.
7/11/2018	n.a.	n.a.	n.a.	n.a.
7/25/2018	n.a.	n.a.	n.a.	n.a.
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	n.a.	n.a.	n.a.	n.a.
9/19/2018	n.a.	n.a.	n.a.	n.a.
10/3/2018	n.a.	n.a.	n.a.	n.a.
10/17/2018	< 0.02000	< 0.02000	< 0.02000	< 0.02000
10/31/2018	n.a.	n.a.	n.a.	n.a.
	Particulate P [PP], mg/L			
Sample Date/Depth (m)	0	2	6	11
5/30/2018	n.a.	n.a.	n.a.	n.a.
6/13/2018	n.a.	n.a.	n.a.	n.a.
7/11/2018	n.a.	n.a.	n.a.	n.a.
7/25/2018	n.a.	n.a.	n.a.	n.a.
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	n.a.	n.a.	n.a.	n.a.
9/19/2018	n.a.	n.a.	n.a.	n.a.
10/3/2018	n.a.	n.a.	n.a.	n.a.
10/17/2018	n.a.	n.a.	n.a.	n.a.
10/31/2018	n.a.	n.a.	n.a.	n.a.
	Total P [TP], mg/L			
Sample Date/Depth (m)	0	2	6	11
5/30/2018	n.a.	n.a.	n.a.	n.a.
6/13/2018	n.a.	n.a.	n.a.	n.a.
7/11/2018	n.a.	n.a.	n.a.	n.a.
7/25/2018	n.a.	n.a.	n.a.	n.a.
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	n.a.	n.a.	n.a.	n.a.
9/19/2018	n.a.	n.a.	n.a.	n.a.
10/3/2018	n.a.	n.a.	n.a.	n.a.
10/17/2018	< 0.02000	< 0.02000	< 0.02000	< 0.02000
10/31/2018	n.a.	n.a.	n.a.	n.a.
	Total P, [TPDW] mg/kg dry wt.			

Sample Date/Depth (m)	0	2	6	11
5/30/2018	n.a.	n.a.	n.a.	n.a.
6/13/2018	n.a.	n.a.	n.a.	n.a.
7/11/2018	n.a.	n.a.	n.a.	n.a.
7/25/2018	n.a.	n.a.	n.a.	n.a.
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	n.a.	n.a.	n.a.	n.a.
9/19/2018	n.a.	n.a.	n.a.	n.a.
10/3/2018	n.a.	n.a.	n.a.	n.a.
10/17/2018	n.a.	n.a.	n.a.	n.a.
10/31/2018	n.a.	n.a.	n.a.	n.a.

The following table 4A shows particulate phosphorous [PP], dissolved phosphorous [DP], total phosphorous [TP], and total phosphorous [TPDW] in mg/kg dry weight for the second year of sampling for Caesar Creek Lake at depths of 0, 2, 6, and 11 meters.

Table 4A – Year 1 fractional phosphorous data sorted by [PP] (mg/L), [DP] (mg/L), [TP] (mg/L), and [TPDW] (mg/kg dry weight) for Caesar Creek Lake at depths of 0, 2, 6, and 11 meters.

	Dissolved P [DP], mg/L			
Sample Date/Depth (m)	0	2	6	11
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	0.0718	0.0730	0.0723	0.0331
4/23/2019	< 0.02000	< 0.02000	0.0041	0.0232
5/7/2019	n.a.	n.a.	n.a.	n.a.
5/22/2019	n.a.	n.a.	n.a.	n.a.
6/5/2019	0.05905	< 0.02000	< 0.02000	< 0.02000
6/19/2019	n.a.	n.a.	n.a.	n.a.
7/2/2019	n.a.	n.a.	n.a.	n.a.
7/16/2019	< 0.02000	0.02192	< 0.02000	< 0.02000
7/30/2019	< 0.02000	< 0.02000	< 0.02000	< 0.02000
8/13/2019	< 0.02000	< 0.02000	< 0.02000	< 0.02000
9/13/2019	< 0.02000	< 0.02000	< 0.02000	< 0.02000
9/26/2019	< 0.02000	< 0.02000	< 0.02000	< 0.02000

	Particulate P [PP], mg/L			
Sample Date/Depth (m)	0	2	6	11
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	n.a.	n.a.	n.a.	n.a.
4/23/2019	< 0.02000	< 0.02000	< 0.02000	< 0.02000
5/7/2019	< 0.02000	< 0.02000	0.0371	0.0315
5/22/2019	0.0494	0.0405	0.0948	0.0316
6/5/2019	< 0.02000	< 0.02000	< 0.02000	< 0.02000
6/19/2019	0.0710	0.1434	0.1109	0.1350
7/2/2019	0.2141	0.2535	0.1242	0.0710
7/16/2019	0.09532	0.08981	0.05402	0.18995
7/30/2019	0.09069	0.09350	< 0.02000	0.27600
8/13/2019	0.10716	0.08228	0.16882	0.20751
9/13/2019	0.12384	0.24360	0.06432	0.25986
9/26/2019	0.17906	0.29577	0.29936	0.37068
	Total P [TP], mg/L			
Sample Date/Depth (m)	0	2	6	11
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	< 0.02000	< 0.02000	< 0.02000	< 0.02000
4/23/2019	< 0.02000	0.0000	0.0041	0.0232
5/7/2019	< 0.02000	< 0.02000	< 0.02000	< 0.02000
5/22/2019	< 0.02000	< 0.02000	< 0.02000	< 0.02000
6/5/2019	0.0591	< 0.02000	< 0.02000	< 0.02000
6/19/2019	< 0.02000	< 0.02000	< 0.02000	< 0.02000
7/2/2019	< 0.02000	< 0.02000	< 0.02000	< 0.02000
7/16/2019	0.0953	0.1117	0.0540	0.1899
7/30/2019	0.0907	0.0935	0.0000	0.2760
8/13/2019	0.1072	0.0823	0.1688	0.2075
9/13/2019	0.1238	0.2436	0.0643	0.2599
9/26/2019	0.1791	0.2958	0.2994	0.3707
	Total P [TPDW], mg/kg dry wt.			
Sample Date/Depth (m)	0	2	6	11
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	n.a.	n.a.	n.a.	n.a.

4/23/2019	n.a.	n.a.	n.a.	n.a.
5/7/2019	n.a.	n.a.	n.a.	n.a.
5/22/2019	n.a.	n.a.	n.a.	n.a.
6/5/2019	n.a.	n.a.	n.a.	n.a.
6/19/2019	n.a.	n.a.	n.a.	n.a.
7/2/2019	n.a.	n.a.	n.a.	n.a.
7/16/2019	n.a.	n.a.	n.a.	n.a.
7/30/2019	n.a.	n.a.	n.a.	n.a.
8/13/2019	n.a.	n.a.	n.a.	n.a.
9/13/2019	n.a.	n.a.	n.a.	n.a.
9/26/2019	n.a.	n.a.	n.a.	n.a.

The following table 5A shows water quality results as obtained by the YSI Professional Plus: NH_4^+-[N] (mg/L), NH_3-[N] (mg/L), Dissolved oxygen, DO (mg/L), and Temperature (°C) for the first year of sampling for Caesar Creek Lake at depths of 0, 2, 6, and 11 meters:

Table 5A– Year 1 water quality data results for the following parameters: NH_4^+-[N] (mg/L), NH_3-[N] (mg/L), Dissolved oxygen, DO (mg/L), and Temperature (°C) for Caesar Creek Lake at depths of 0, 2, 6, and 11 meters.

	[NH_4^+-N] (mg/L)			
Sample Date/Depth (m)	0	2	6	11
5/30/2018	0.31	0.34	0.40	0.34
6/13/2018	0.29	0.29	0.42	0.38
7/11/2018	0.16	0.31	0.19	0.22
7/25/2018	0.25	0.39	0.36	0.44
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	0.13	0.11	0.13	0.45
9/19/2018	0.05	0.05	0.06	0.16
10/3/2018	0.20	0.22	0.24	0.40
10/17/2018	0.17	0.18	0.15	0.25
10/31/2018	n.a.	n.a.	n.a.	n.a.
	[NH_3–N] (mg/L)			
Sample Date/Depth (m)	0	2	6	11
5/30/2018	0.04	0.04	0.00	0.00

6/13/2018	0.02	0.02	0.01	0.00
7/11/2018	0.03	0.05	0.02	0.00
7/25/2018	0.03	0.04	0.01	0.00
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	0.03	0.03	0.01	0.00
9/19/2018	0.02	0.02	0.00	0.00
10/3/2018	0.02	0.02	0.01	0.01
10/17/2018	0.01	0.01	0.01	0.01
10/31/2018	n.a.	n.a.	n.a.	n.a.
	Dissolved oxygen, DO (mg/L)			
Sample Date/Depth (m)	0	2	6	11
5/30/2018	10.28	8.34	3.07	6.56
6/13/2018	6.44	6.17	2.54	3.12
7/11/2018	7.37	6.64	3.54	1.44
7/25/2018	7.87	7.45	3.88	2.51
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	7.47	7.25	3.97	1.13
9/19/2018	10.52	8.57	2.23	1.88
10/3/2018	10.27	8.48	6.31	3.12
10/17/2018	6.14	5.83	5.39	6.56
10/31/2018	n.a.	n.a.	n.a.	n.a.
	Temperature (°C)			
Sample Date/Depth (m)	0	2	6	11
5/30/2018	26.50	26.40	17.80	11.80
6/13/2018	24.10	24.00	21.90	12.30
7/11/2018	27.50	27.30	26.30	16.00
7/25/2018	26.70	26.10	25.30	17.90
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	26.80	26.70	25.40	18.20
9/19/2018	26.40	25.60	23.50	20.40
10/3/2018	22.80	22.40	21.90	20.50
10/17/2018	18.50	17.90	17.80	18.20
10/31/2018	n.a.	n.a.	n.a.	n.a.

The following table 6A shows water quality results as obtained by the YSI Professional Plus: NH_4^+-[N] (mg/L), NH_3-[N] (mg/L), Dissolved oxygen, DO (mg/L), and Temperature (°C) for the second year of sampling for Caesar Creek Lake at depths of 0, 2, 6, and 11 meters.

Table 6A – Year 2 water quality data results for the following parameters: NH_4^+-[N] (mg/L), NH_3-[N] (mg/L), Dissolved oxygen, DO (mg/L), and Temperature (°C) for Caesar Creek Lake at depths of 0, 2, 6, and 11 meters.

	[NH_4^+-N] (mg/L)			
Sample Date/Depth (m)	**0**	**2**	**6**	**11**
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	0.31	0.25	0.22	0.24
4/23/2019	0.47	0.31	0.28	0.53
5/7/2019	0.36	0.27	0.38	0.40
5/22/2019	0.56	0.27	0.26	0.26
6/5/2019	0.34	0.26	0.30	0.30
6/19/2019	0.49	0.30	0.38	0.28
7/2/2019	0.15	0.14	0.28	0.22
7/16/2019	0.22	0.22	0.37	0.00
7/30/2019	0.30	0.23	0.38	0.74
8/13/2019	0.34	0.24	0.30	0.90
9/13/2019	0.36	0.24	0.40	1.16
9/26/2019	0.31	0.20	0.21	1.21
	[NH_3–N] (mg/L)			
Sample Date/Depth (m)	**0**	**2**	**6**	**11**
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	0.00	0.00	0.00	0.00
4/23/2019	0.00	0.00	0.00	0.00
5/7/2019	0.02	0.03	0.01	0.01
5/22/2019	0.01	0.00	0.00	0.00
6/5/2019	0.01	0.01	0.01	0.00
6/19/2019	0.00	0.00	0.00	0.00
7/2/2019	0.03	0.03	0.01	0.00
7/16/2019	0.02	0.02	0.00	0.50
7/30/2019	0.00	0.00	0.01	0.00

8/13/2019	0.00	0.00	0.00	0.00
9/13/2019	0.00	0.00	0.00	0.00
9/26/2019	0.00	0.00	0.00	0.00
	Dissolved oxygen, DO (mg/L)			
Sample Date/Depth (m)	0	2	6	11
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	12.06	11.97	10.69	10.52
4/23/2019	13.91	13.30	10.12	9.69
5/7/2019	16.38	14.61	9.26	8.93
5/22/2019	9.20	8.99	7.64	5.06
6/5/2019	10.93	9.54	5.33	2.94
6/19/2019	12.79	8.29	6.40	2.99
7/2/2019	12.02	11.25	3.86	1.74
7/16/2019	8.41	5.94	0.81	1.01
7/30/2019	7.34	7.35	1.86	1.96
8/13/2019	8.49	7.72	5.11	1.77
9/13/2019	9.56	9.36	3.70	2.22
9/26/2019	6.92	7.31	6.84	2.02
	Temperature (°C)			
Sample Date/Depth (m)	0	2	6	11
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	12.90	12.40	11.80	8.70
4/23/2019	14.30	13.90	11.60	10.80
5/7/2019	20.10	19.10	15.40	13.80
5/22/2019	19.00	19.10	18.10	13.60
6/5/2019	22.80	22.50	20.90	14.70
6/19/2019	24.10	22.99	21.63	16.43
7/2/2019	27.50	27.30	23.40	21.50
7/16/2019	27.80	27.90	24.30	20.80
7/30/2019	26.90	26.80	25.70	21.20
8/13/2019	26.60	26.41	26.26	20.44
9/13/2019	25.91	25.90	23.83	20.75
9/26/2019	23.45	23.39	23.37	19.40

The following table 7A shows nitrate and phosphate concentrations in mg/L units for Anderson Fork (AF), Buck Run (BR), Caesar Creek (CAC), and Turkey Run (TR) for the first year of sampling.

Table 7A – Year 1 nitrate and phosphate results in mg/L for Anderson Fork (AF), Buck Run (BR), Caesar Creek (CAC), and Turkey Run (TR).

	Nitrate (as NO_3^-, mg/L)			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
5/30/2018	n.a.	n.a.	n.a.	n.a.
6/13/2018	n.a.	n.a.	n.a.	n.a.
7/11/2018	n.a.	n.a.	n.a.	n.a.
7/25/2018	n.a.	n.a.	n.a.	n.a.
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	n.a.	n.a.	n.a.	n.a.
9/19/2018	n.a.	n.a.	n.a.	n.a.
10/3/2018	n.a.	n.a.	n.a.	n.a.
10/17/2018	n.a.	n.a.	n.a.	n.a.
10/31/2018	n.a.	n.a.	n.a.	n.a.
	Nitrite (as NO_2^-, mg/L)			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
5/30/2018	n.a.	n.a.	n.a.	n.a.
6/13/2018	n.a.	n.a.	n.a.	n.a.
7/11/2018	n.a.	n.a.	n.a.	n.a.
7/25/2018	n.a.	n.a.	n.a.	n.a.
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	n.a.	n.a.	n.a.	n.a.
9/19/2018	n.a.	n.a.	n.a.	n.a.
10/3/2018	n.a.	n.a.	n.a.	n.a.
10/17/2018	n.a.	n.a.	n.a.	n.a.
10/31/2018	n.a.	n.a.	n.a.	n.a.
	Phosphate (as PO_4^{3-}, mg/L)			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
5/30/2018	n.a.	n.a.	n.a.	n.a.
6/13/2018	n.a.	n.a.	n.a.	n.a.
7/11/2018	n.a.	n.a.	n.a.	n.a.
7/25/2018	n.a.	n.a.	n.a.	n.a.
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	n.a.	n.a.	n.a.	n.a.
9/19/2018	n.a.	n.a.	n.a.	n.a.

10/3/2018	n.a.	n.a.	n.a.	n.a.
10/17/2018	n.a.	n.a.	n.a.	n.a.
10/31/2018	n.a.	n.a.	n.a.	n.a.

The following table 8A shows nitrate and phosphate concentrations in mg/L for Anderson Fork (AF), Buck Run (BR), Caesar Creek (CAC), and Turkey Run (TR) for the first year of sampling.

Table 8A – Year 2 nitrate and phosphate results in mg/L for Anderson Fork (AF), Buck Run (BR), Caesar Creek (CAC), and Turkey Run (TR).

	Nitrate (as NO_3^-, mg/L)			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
1/23/2019	9.371	4.689	8.834	2.274
4/9/2019	0.629	-0.201	2.419	1.154
4/23/2019	23.395	4.867	20.750	5.199
5/7/2019	26.435	9.167	4.899	1.481
5/22/2019	9.232	1.621	6.158	5.203
6/5/2019	15.715	13.279	13.426	25.751
6/19/2019	n.a.	n.a.	n.a.	n.a.
7/2/2019	10.125	8.074	7.440	6.571
7/16/2019	5.103	5.432	5.285	2.613
7/30/2019	0.884	< LOD	4.878	< LOD
8/13/2019	0.470	1.786	5.213	3.088
9/13/2019	7.943	< LOD	2.802	< LOD
9/26/2019	0.049	0.020	0.159	0.062
Table 8	Nitrite (as NO_2^-, mg/L)			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
1/23/2019	< LOD	< LOD	< LOD	< LOD
4/9/2019	< LOD	< LOD	< LOD	< LOD
4/23/2019	< LOD	< LOD	< LOD	< LOD
5/7/2019	< LOD	< LOD	< LOD	0.083
5/22/2019	< LOD	< LOD	< LOD	< LOD
6/5/2019	0.036	0.367	2.591	5.096
6/19/2019	n.a.	n.a.	n.a.	n.a.

7/2/2019	< LOD	< LOD	< LOD	< LOD
7/16/2019	< LOD	< LOD	< LOD	< LOD
7/30/2019	< LOD	< LOD	< LOD	< LOD
8/13/2019	< LOD	< LOD	< LOD	< LOD
9/13/2019	< LOD	< LOD	< LOD	< LOD
9/26/2019	< LOD	< LOD	< LOD	< LOD

Table 8	Phosphate (as PO_4^{3-}, mg/L)			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
1/23/2019	< LOD	< LOD	< LOD	< LOD
4/9/2019	< LOD	< LOD	< LOD	< LOD
4/23/2019	< LOD	< LOD	< LOD	< LOD
5/7/2019	< LOD	< LOD	< LOD	< LOD
5/22/2019	< LOD	< LOD	< LOD	< LOD
6/5/2019	< LOD	< LOD	< LOD	< LOD
6/19/2019	n.a.	n.a.	n.a.	n.a.
7/2/2019	< LOD	< LOD	< LOD	< LOD
7/16/2019	< LOD	< LOD	< LOD	< LOD
7/30/2019	< LOD	< LOD	< LOD	< LOD
8/13/2019	< LOD	< LOD	< LOD	< LOD
9/13/2019	< LOD	< LOD	< LOD	< LOD
9/26/2019	< LOD	< LOD	< LOD	< LOD

The following table 9A shows particulate phosphorous [PP] (mg/L), dissolved phosphorous [DP] (mg/L), total phosphorous [TP] (mg/L), and total phosphorous [TPDW] in mg/kg dry weight for Anderson Fork (AF), Buck Run (BR), Caesar Creek (CAC), and Turkey Run (TR)..

Table 9A – Year 1 fractional phosphorous data sorted by [PP] (mg/L), [DP] (mg/L), [TP] (mg/L), and [TPDW] (mg/kg dry weight) for Anderson Fork (AF), Buck Run (BR), Caesar Creek (CAC), and Turkey Run (TR).

	Dissolved P [DP], mg/L			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
5/30/2018	n.a.	n.a.	n.a.	n.a.
6/13/2018	n.a.	n.a.	n.a.	n.a.
7/11/2018	n.a.	n.a.	n.a.	n.a.

7/25/2018	n.a.	n.a.	n.a.	n.a.
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	n.a.	n.a.	n.a.	n.a.
9/19/2018	n.a.	n.a.	n.a.	n.a.
10/3/2018	n.a.	n.a.	n.a.	n.a.
10/17/2018	n.a.	n.a.	n.a.	n.a.
10/31/2018	n.a.	n.a.	n.a.	n.a.
Table 9	Particulate P [PP], mg/L			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
5/30/2018	n.a.	n.a.	n.a.	n.a.
6/13/2018	n.a.	n.a.	n.a.	n.a.
7/11/2018	n.a.	n.a.	n.a.	n.a.
7/25/2018	n.a.	n.a.	n.a.	n.a.
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	n.a.	n.a.	n.a.	n.a.
9/19/2018	n.a.	n.a.	n.a.	n.a.
10/3/2018	n.a.	n.a.	n.a.	n.a.
10/17/2018	n.a.	n.a.	n.a.	n.a.
10/31/2018	n.a.	n.a.	n.a.	n.a.
Table 9	Total P, mg/L			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
5/30/2018	n.a.	n.a.	n.a.	n.a.
6/13/2018	n.a.	n.a.	n.a.	n.a.
7/11/2018	n.a.	n.a.	n.a.	n.a.
7/25/2018	n.a.	n.a.	n.a.	n.a.
7/27/2018	n.a.	n.a.	n.a.	n.a.
8/8/2018	n.a.	n.a.	n.a.	n.a.
9/19/2018	n.a.	n.a.	n.a.	n.a.
10/3/2018	n.a.	n.a.	n.a.	n.a.
10/17/2018	n.a.	n.a.	n.a.	n.a.
10/31/2018	n.a.	n.a.	n.a.	n.a.
Table 9	Total P, mg/kg dry wt.			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
5/30/2018	n.a.	n.a.	n.a.	n.a.
6/13/2018	n.a.	n.a.	n.a.	n.a.

7/11/2018	n.a.	n.a.	n.a.	n.a.
7/25/2018	n.a.	n.a.	n.a.	n.a.
7/27/2018	71.11705	187.14642	390.96432	n.a.
8/8/2018	n.a.	n.a.	n.a.	n.a.
9/19/2018	n.a.	n.a.	n.a.	n.a.
10/3/2018	n.a.	n.a.	n.a.	n.a.
10/17/2018	n.a.	n.a.	n.a.	n.a.
10/31/2018	n.a.	n.a.	n.a.	n.a.

The following table 10A shows particulate phosphorous [PP], dissolved phosphorous [DP], total phosphorous [TP], and total phosphorous [TPDW] in mg/kg dry weight for the second year of sampling for Anderson Fork (AF), Buck Run (BR), Caesar Creek (CAC), and Turkey Run (TR).

Table 10A – Year 1 fractional phosphorous data sorted by [PP] (mg/L), [DP] (mg/L), [TP] (mg/L), and [TPDW] (mg/kg dry weight) for Anderson Fork (AF), Buck Run (BR), Caesar Creek (CAC), and Turkey Run (TR).

	Dissolved P [DP], mg/L			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
1/23/2019	<0.02000 mg/L	<0.02000 mg/L	0.0220	<0.02000 mg/L
4/9/2019	<0.02000 mg/L	<0.02000 mg/L	<0.02000 mg/L	<0.02000 mg/L
4/23/2019	0.0886	<0.02000 mg/L	<0.02000 mg/L	<0.02000 mg/L
5/7/2019	n.a.	n.a.	n.a.	n.a.
5/22/2019	n.a.	n.a.	n.a.	n.a.
6/5/2019	0.02245	0.13594	< 0.02000 mg/L	0.09293
6/19/2019	n.a.	n.a.	n.a.	n.a.
7/2/2019	n.a.	n.a.	n.a.	n.a.
7/16/2019	<0.02000 mg/L	<0.02000 mg/L	<0.02000 mg/L	<0.02000 mg/L
7/30/2019	<0.02000 mg/L	<0.02000 mg/L	<0.02000 mg/L	<0.02000 mg/L
8/13/2019	<0.02000 mg/L	0.09066	<0.02000 mg/L	0.10955

9/13/2019	0.02873	<0.02000 mg/L	0.02545	<0.02000 mg/L
9/26/2019	<0.02000 mg/L	<0.02000 mg/L	< 0.02000 mg/L	<0.02000 mg/L
	Particulate P [PP], mg/L			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	n.a.	n.a.	n.a.	n.a.
4/23/2019	<0.02000 mg/L	<0.02000 mg/L	<0.02000 mg/L	<0.02000 mg/L
5/7/2019	<0.02000 mg/L	<0.02000 mg/L	n.a.	n.a.
5/22/2019	0.0755	<0.02000 mg/L	0.1299	<0.02000 mg/L
6/5/2019	0.07775	0.09675	0.03387	0.88287
6/19/2019	n.a.	n.a.	n.a.	n.a.
7/2/2019	0.1117	0.0499	0.0556	<0.02000 mg/L
7/16/2019	0.06083	0.02025	0.07130	<0.02000 mg/L
7/30/2019	0.38940	0.23846	0.26527	0.03885
8/13/2019	0.59338	2.04115	0.71620	1.99568
9/13/2019	0.15429	0.12252	0.27222	0.09185
9/26/2019	0.27225	0.26075	0.32781	0.40778
	Total P, mg/L			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
1/23/2019	< 0.02000 mg/L	< 0.02000 mg/L	< 0.02000 mg/L	< 0.02000 mg/L
4/9/2019	< 0.02000 mg/L	< 0.02000 mg/L	< 0.02000 mg/L	< 0.02000 mg/L
4/23/2019	< 0.02000 mg/L	< 0.02000 mg/L	< 0.02000 mg/L	< 0.02000 mg/L
5/7/2019	< 0.02000 mg/L	< 0.02000 mg/L	< 0.02000 mg/L	< 0.02000 mg/L
5/22/2019	< 0.02000 mg/L	< 0.02000 mg/L	< 0.02000 mg/L	< 0.02000 mg/L
6/5/2019	0.1002	0.2327	0.0339	0.9758
6/19/2019	n.a.	n.a.	n.a.	n.a.
7/2/2019	< 0.02000 mg/L	< 0.02000 mg/L	< 0.02000 mg/L	< 0.02000 mg/L

7/16/2019	0.0608	0.0203	0.0713	< 0.02000
7/30/2019	0.3894	0.2385	0.2653	0.0388
8/13/2019	0.5934	2.1318	0.7162	2.1052
9/13/2019	0.1830	0.1225	0.2977	0.0919
9/26/2019	0.2723	0.2608	0.3278	0.4078
	Total P, mg/kg dry wt.			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	n.a.	n.a.	n.a.	n.a.
4/23/2019	n.a.	n.a.	n.a.	n.a.
5/7/2019	n.a.	n.a.	n.a.	n.a.
5/22/2019	n.a.	n.a.	n.a.	n.a.
6/5/2019	n.a.	n.a.	n.a.	n.a.
6/19/2019	n.a.	n.a.	n.a.	n.a.
7/2/2019	n.a.	n.a.	n.a.	n.a.
7/16/2019	n.a.	n.a.	n.a.	n.a.
7/30/2019	n.a.	n.a.	n.a.	n.a.
8/13/2019	n.a.	n.a.	n.a.	n.a.
9/13/2019	n.a.	n.a.	n.a.	n.a.
9/26/2019	n.a.	n.a.	n.a.	n.a.

The following table 11A shows water quality results as obtained by the YSI Professional Plus: NH_4^+-[N] (mg/L), NH_3-[N] (mg/L), Dissolved oxygen, DO (mg/L), and Temperature (°C) for the first year of sampling for Anderson Fork (AF), Buck Run (BR), Caesar Creek (CAC), and Turkey Run (TR).

Table 11A – Year 1 water quality data results for the following parameters: NH_4^+ - [N] (mg/L), NH_3-[N] (mg/L), Dissolved oxygen, DO (mg/L), and Temperature (°C) for Anderson Fork (AF), Buck Run (BR), Caesar Creek (CAC), and Turkey Run (TR).

	NH_4^+-[N] (mg/L)			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
5/30/2018	n.a.	n.a.	n.a.	n.a.
6/13/2018	n.a.	n.a.	n.a.	n.a.
7/11/2018	n.a.	n.a.	n.a.	n.a.

7/25/2018	n.a.	n.a.	n.a.	n.a.
7/27/2018	0.10	0.09	0.09	n.a.
8/8/2018	n.a.	n.a.	n.a.	n.a.
9/19/2018	n.a.	n.a.	n.a.	n.a.
10/3/2018	n.a.	n.a.	n.a.	n.a.
10/17/2018	n.a.	n.a.	n.a.	n.a.
10/31/2018	n.a.	n.a.	n.a.	n.a.
	NH_3–[N] (mg/L)			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
5/30/2018	n.a.	n.a.	n.a.	n.a.
6/13/2018	n.a.	n.a.	n.a.	n.a.
7/11/2018	n.a.	n.a.	n.a.	n.a.
7/25/2018	n.a.	n.a.	n.a.	n.a.
7/27/2018	0.00	0.00	0.02	n.a.
8/8/2018	n.a.	n.a.	n.a.	n.a.
9/19/2018	n.a.	n.a.	n.a.	n.a.
10/3/2018	n.a.	n.a.	n.a.	n.a.
10/17/2018	n.a.	n.a.	n.a.	n.a.
10/31/2018	n.a.	n.a.	n.a.	n.a.
	Dissolved oxygen, DO (mg/L)			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
5/30/2018	n.a.	n.a.	n.a.	n.a.
6/13/2018	n.a.	n.a.	n.a.	n.a.
7/11/2018	n.a.	n.a.	n.a.	n.a.
7/25/2018	n.a.	n.a.	n.a.	n.a.
7/27/2018	6.18	7.04	6.22	n.a.
8/8/2018	n.a.	n.a.	n.a.	n.a.
9/19/2018	n.a.	n.a.	n.a.	n.a.
10/3/2018	n.a.	n.a.	n.a.	n.a.
10/17/2018	n.a.	n.a.	n.a.	n.a.
10/31/2018	n.a.	n.a.	n.a.	n.a.
	Temperature (°C)			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
5/30/2018	n.a.	n.a.	n.a.	n.a.
6/13/2018	n.a.	n.a.	n.a.	n.a.
7/11/2018	n.a.	n.a.	n.a.	n.a.

7/25/2018	n.a.	n.a.	n.a.	n.a.
7/27/2018	25.80	28.20	27.00	n.a.
8/8/2018	n.a.	n.a.	n.a.	n.a.
9/19/2018	n.a.	n.a.	n.a.	n.a.
10/3/2018	n.a.	n.a.	n.a.	n.a.
10/17/2018	n.a.	n.a.	n.a.	n.a.
10/31/2018	n.a.	n.a.	n.a.	n.a.

The following table 12A shows water quality results as obtained by the YSI Professional Plus: NH_4^+-[N] (mg/L), NH_3-[N] (mg/L), Dissolved oxygen, DO (mg/L), and Temperature (°C) for the second year of sampling for Anderson Fork (AF), Buck Run (BR), Caesar Creek (CAC), and Turkey Run (TR).

Table 12A – Year 2 water quality data results for the following parameters: NH_4^+-[N] (mg/L), NH_3-[N] (mg/L), Dissolved oxygen, DO (mg/L), and Temperature (°C) for Anderson Fork (AF), Buck Run (BR), Caesar Creek (CAC), and Turkey Run (TR).

	NH_4^+-[N] (mg/L)			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	0.19	0.15	0.17	0.16
4/23/2019	0.28	0.21	0.23	0.25
5/7/2019	0.21	0.20	0.23	0.28
5/22/2019	0.19	0.16	0.17	0.21
6/5/2019	0.19	0.34	0.18	0.75
6/19/2019	n.a.	n.a.	n.a.	n.a.
7/2/2019	0.11	0.14	0.13	0.16
7/16/2019	0.17	0.21	0.22	0.28
7/30/2019	0.21	0.27	0.19	0.35
8/13/2019	0.34	0.64	0.31	0.84
9/13/2019	0.33	0.30	0.33	0.37
9/26/2019	0.20	0.11	0.16	0.20
Table 12	NH_3 – [N] (mg/L)			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
1/23/2019	n.a.	n.a.	n.a.	n.a.

4/9/2019	0.01	0.01	0.00	0.00
4/23/2019	0.01	0.01	0.00	0.01
5/7/2019	0.01	0.01	0.01	0.00
5/22/2019	0.00	0.01	0.00	0.00
6/5/2019	0.00	0.01	0.00	0.01
6/19/2019	n.a.	n.a.	n.a.	n.a.
7/2/2019	0.01	0.01	0.01	0.01
7/16/2019	0.01	0.01	0.01	0.01
7/30/2019	0.01	0.01	0.01	0.01
8/13/2019	0.00	0.00	0.00	0.00
9/13/2019	0.00	0.00	0.00	0.00
9/26/2019	0.00	0.00	0.00	0.00

Table 12	Dissolved oxygen, DO (mg/L)			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	11.56	11.80	12.60	12.95
4/23/2019	10.34	10.69	10.44	10.33
5/7/2019	10.46	12.02	10.34	11.30
5/22/2019	10.18	16.95	10.04	11.78
6/5/2019	7.80	8.57	7.93	8.35
6/19/2019	n.a.	n.a.	n.a.	n.a.
7/2/2019	7.76	8.35	8.36	8.21
7/16/2019	8.67	11.72	9.36	8.64
7/30/2019	6.18	9.75	7.68	9.24
8/13/2019	7.55	8.77	7.78	8.59
9/13/2019	12.36	4.92	9.78	9.36
9/26/2019	5.14	5.10	8.83	8.50

Table 12	Temperature (°C)			
Sample Date/Trib.	Anderson Fork (AF)	Buck Run (BR)	Caesar Creek (CAC)	Turkey Run (TR)
1/23/2019	n.a.	n.a.	n.a.	n.a.
4/9/2019	15.20	16.10	15.30	15.30
4/23/2019	16.10	16.60	15.40	15.70
5/7/2019	20.00	18.60	18.00	18.20
5/22/2019	17.00	18.20	16.10	15.80
6/5/2019	19.80	18.80	19.10	18.00
6/19/2019	n.a.	n.a.	n.a.	n.a.

7/2/2019	24.40	24.40	24.20	23.10
7/16/2019	26.10	24.40	24.40	23.40
7/30/2019	24.60	23.00	22.90	22.20
8/13/2019	22.48	21.50	21.28	21.51
9/13/2019	27.37	22.52	26.08	22.32
9/26/2019	21.79	18.30	20.51	17.82

The following Table 13A is a summary of [MC], µg/L for LT2001 (untreated WWD treatment facility water at tap), RS001 (samples directly taken from Caesar Creek Lake), and L-1, L-2, and L-3 sites were used by the Ohio EPA during the 2017 HAB. The L-1, L-2, and L-3 sites had GPS coordinates of (39.486160, -84.059270), (39.506790, -84.010670), and (39.538230, -83.990990) respectively. [MC], µg/L concentrations are reported as averages of daily values.

Table 13A - Summary of [MC] for LT2001 (untreated WWD treatment facility water at tap), RS001 (samples directly taken from Caesar Creek Lake), and L-1, L-2, and L-3 sites were used by the Ohio EPA during the 2017 HAB. The L-1, L-2, and L-3 sites had GPS coordinates of (39.486160, -84.059270), (39.506790, -84.010670), and (39.538230, -83.990990) respectively. [MC], µg/L concentrations are reported as averages of daily values. [20]

Date	Sample Site	[MC], µg/L
05/25/18	L-1	0.231
05/26/18	LT2001	0.174
05/31/18	LT2001	0.117
06/06/18	L-2	0.000
06/07/18	LT2001	0.127
06/14/18	LT2001	0.163
06/19/18	L-2	0.000
06/21/18	LT2001	0.036
07/10/18	L-1	0.000
07/19/18	LT2001	0.000
07/24/18	LT2001	0.192
07/25/18	LT2001	0.086
08/02/18	LT2001	0.131
08/07/18	LT2001	0.100
08/09/18	LT2001	0.000
08/16/18	LT2001	0.022
08/22/18	L-2	0.000
08/23/18	LT2001	0.113
08/30/18	LT2001	0.132

09/06/18	LT2001	0.074
09/11/18	L-1	0.000
09/13/18	LT2001	0.069
09/19/18	LT2001	0.087
09/27/18	LT2001	0.085
10/02/18	LT2001	0.075
10/04/18	LT2001	0.039
10/11/18	LT2001	0.139
10/18/18	LT2001	0.090
10/25/18	LT2001	0.057
11/01/18	LT2001	0.148
11/15/18	LT2001	0.206
11/29/18	LT2001	0.109
04/17/19	RS001	0.005
05/02/19	LT2001	0.057
05/09/19	LT2001	0.078
05/16/19	LT2001	0.146
05/22/19	RS001	0.110
05/30/19	RS001	0.170
06/06/19	LT2001	0.365
06/13/19	LT2001	0.809
06/19/19	RS001	2.608
06/26/19	RS001	1.764
07/02/19	RS001	3.799
07/10/19	RS001	3.198
07/17/19	RS001	1.381
07/25/19	RS001	1.372
07/31/19	RS001	0.625
08/08/19	RS001	0.501
08/13/19	RS001	0.276
08/22/19	RS001	0.178
08/30/19	RS001	0.188
09/05/19	RS001	0.214
09/12/19	RS001	0.225
09/27/19	LT2001	0.105
10/09/19	LT2001	0.000
10/10/19	LT2001	0.103
10/17/19	LT2001	0.150
10/24/19	LT2001	0.160

VIII. APPENDIX B – Standard Operating Procedures (SOPs)

The following is a collection of standard operating procedures (SOPs) used in the study. They are organized in numerical order by SOP number (e.g. SOP 4.5, SOP 5.7, etc.).

STANDARD OPERATING PROCEDURE 4.5

DETERMINATION OF INORGANIC ANIONS BY ION CHROMATOGRAPHY (IC)

IN SURFACE AND GROUND WATER SAMPLES

BY EPA METHOD 300.1

Revised September 29, 2015

By

Jessica McKinley and Audrey McGowin, Ph.D.

Approved: _____

Audrey McGowin, Ph.D.

1. SCOPE AND APPLICATION

This method utilizes EPA Method 300.1 and ion chromatography to determine selected anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-}, and SO_4^{2-}) in water samples. A small volume of the sample solution is injected into the ion chromatograph (IC) into a flowing stream of eluent (carbonate-bicarbonate) solution. Detection is achieved using a suppressor column and a conductivity detector. Anion identification is based on the comparison of analyte signal peak retention times relative to those of known standards. Quantitation is accomplished by measuring the peak area and comparing it to a calibration curve established from known standards. In addition, there is a maximum holding time associated with these ions.

2. SUMMARY OF METHOD

This method applies to sample analysis by IC for F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-}, and SO_4^{2-} in surface water and groundwater.

3. HEALTH AND SAFETY

The analyst must assume that all surface water samples are potentially contaminated and should be treated accordingly. Personal protective equipment (PPE) should be worn at all times while in the lab. This includes lab coat, nitrile gloves, and safety glasses, in addition to long pants and closed toes shoes. Expired water samples and anion standards can be poured down the drain because all anions are at trace levels.

4. EQUIPMENT AND SUPPLIES

- 4.1 A Dionex ICS-1600 Ion Chromatograph (IC) system that includes the following components and accessories:
  - 4.1.1 Dionex IonPac AS22 anion-exchange column (4 x 250 mm). This column has a particle diameter of 65 μm. The substrate is polyvinylbenzyl ammonium cross-linked with divinylbenzene (55%). The functional group is alkanol quaternary ammonium with ultralow hydrophobicity.
  - 4.1.2 Dionex AG22 guard column (4 x 50 mm). The guard column substrate is also polyvinylbenzyl ammonium cross-linked with divinylbenzene (55%) with a particle size of 110 μm.
  - 4.1.3 ASRS®300 4-mm anion suppressor column
  - 4.1.4 AS40 automated sampler with a 25-μL sample loop
  - 4.1.5 Autosampler racks
  - 4.1.6 0.5 mL Dionex polyvials and filter caps
- 4.2 Pre-cleaned 50-mL or 100-mL beakers for weighing calibration solutions
- 4.3 Pre-cleaned high-density polyethylene (HDPE) bottles (125- or 250-mL) for samples and storage of calibration solutions
- 4.4 Analytical balance with ±0.1 mg sensitivity for calibration solution preparation

4.5 Pastor pipettes and pastor pipette bulbs
4.6 Disposable 10-mL BD syringe (Latex Free Luer-LokTM)
4.7 Disposable 0.2-µm pore size syringe filter (Whatman ZC)
4.8 1000-mL volumetric flask for preparation of eluent solution

5. REAGENTS AND STANDARDS

5.1 ASTM Type I (18 MΩ) water or high quality filtered deionized water for preparing calibration standards and diluting samples, as needed.
5.2 Eluent: 4.5 mM Na_2CO_3/1.4 mM $NaHCO_3$ prepared from stock solution
5.3 Dionex Combined Seven Anion Standard 1: 50 mL (Cat. No. 056933) that contains 20 mg/L F^-, 30 mg/L Cl^-, 100 mg/L NO_2^-, 100 mg/L Br^-, 100 mg/L NO_3^-, 150 mg/L PO_4^{3-}, and 150 mg/L SO_4^{2-}.
5.4 Quality Control Sample (QCS): Alltech Anion Mix 1 (Cat. No. 269106) which contains 1 µg/mL F^- and 5 µg/mL each of Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-}, and SO_4^{2-}.

6. SAMPLE COLLECTION AND PRESERVATION

6.1 Sample collection and preservation must be performed in accordance with SOP 3. Samples should be collected in pre-cleaned 125-mL HDPE bottles and immediately placed in a cooler and cooled to 4 °C immediately upon collection before being transported to a refrigerator in the laboratory and kept at 4 °C for up to 48 h.

6.2 Sample holding times from EPA Method 300.1 are bromide, chloride, fluoride, and sulfate 28 days. For nitrate, nitrite, and ortho-phosphate the holding time is only 48 h.

7. QUALITY CONTROL

To assure minimum QC, SOP 2 regarding equipment (sample bottles and autosampler vials) cleaning for IC analysis should be followed. New standards need to be prepared within 24 hours of analyzing the samples since the low standards of nitrite will oxidize significantly reducing their concentration and raising the limit of detection. Samples should be injected twice for duplicate analyses. A quality control check solution (QCS) will be run with each set of samples. A standard solution will also be run at least once every 10 samples.

8. PROCEDURES

8.1 PREPARATION OF SAMPLE VIALS

Clean autosampler vials according to SOP 2. They soak for at least 24 hours before the final rinses and use.

8.2 PREPARATION OF CARBONATE-BICARBONATE ELUENT

The eluent for anion IC analysis is a solution of 4.5 mM Na_2CO_3/1.4 mM $NaHCO_3$. Pipette 20.00 mL of AS22 Eluent Concentrate into a clean 2-L volumetric flask and dilute to two liters.

8.2 ASSESSMENT OF LABORATORY PERFORMANCE

8.2.1 Laboratory Reagent Blank (LRB) – Use high quality water (18 MΩ) as the blank solution because all standards and diluted samples must be prepared using ASTM I or high-quality filtered (0.22-μm pores) DI water. An LRB must be analysis with each sample batch. If the LRB exceed the method detection limit (MDL), contamination is suspected and corrective action must be taken.

8.2.2 Method Detection Limit (MDL) – MDLs are determined by analyzing the reagent water blank that has been fortified to a concentration that is three to five times the estimated detection limit. For this analysis, the next to the lowest standard solution can be used to determine the MDL. Analyze seven aliquots of this solution that has been through the entire analytical process (filtering, dilutions, calculations, etc.). Calculate the MDL using the following equation:

MDL = (t) x (S)

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates].
S = standard deviation of the replicate analyses.

8.2.3 Field Duplicates (FD) – Two samples that were collected at the same site under identical circumstances that are used to indicate precision for sample collection, preservation and storage, and sample preparation procedures.

8.2.4 Quality Control Sample (QCS) – This is a sample with known anion concentrations that is analyzed alongside field samples to ensure that instrument performance is acceptable. The determined concentrations should be within ±15% of the stated values for performance to be acceptable. If the performance is determined to be unacceptable, the problem must be identified and corrected before proceeding with further analysis of samples.

8.2.6 Minimum Reporting Level (MRL) – The minimum concentration of each analyte that can be reported. This is usually the concentration of the lowest Calibration Standard that is within the Linear Calibration Range.

9.0 CALIBRATION AND STANDARDIZATION

Prepare new analytical standards before each analysis. The Linear Calibration Range (LCR) should cover the concentration range of the field samples. It may not extent over two orders of magnitude. If it does, then two separate calibration curves should be prepared. A minimum of five calibration standards should be analyzed for a calibration curve that extends over two orders of magnitude. Refer to Table 1. All mass measurements must be done using an analytical balance and weighed to the nearest 0.0001 g.

Parts-per-million (ppm) can be in units of mg/L or mg/kg so but it must be defined. The Dionex Combined Seven Anion Standard 1 (50 mL, Cat. No. 056933) contains 20 mg/L F^-, 30 mg/L Cl^-, 100 mg/L NO_2^-, 100 mg/L Br^-, 100 mg/L NO_3^-, 150 mg/L PO_4^{3-}, and 150 mg/L SO_4^{2-}. These are given in concentration units of mg/L. If you dismiss the error that results from the fact that 1.000 mL of water has a mass of 0.9982 g at 20 °C, then standard solutions can be prepared by weighing an aliquot of primary standard solution in mg/L and diluting with water on an analytical balance to have concentration in units of mg/kg (ppm).

Table 1. Calibration Standard Preparation for IC analysis. Concentration units are mg/kg (ppm).

		F^-	Cl^-	NO_2^-	Br^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
	Initial Conc. (mg/L)	20.0	30.0	100	100	100	150	150
Standard 1	5 g Seven Anion Stock + 5 g H_2O	10.0	15.0	50.0	50.0	50.0	75.0	75.0
Standard 2	1 g Standard 1 + 9 g H_2O	1.00	1.50	5.00	5.00	5.00	7.50	7.50
Standard 3	1 g Standard 1 + 24 g H_2O	0.400	0.600	2.00	2.00	2.00	3.00	3.00
Standard 4	5 g Standard 3 + 5 g H_2O	0.200	0.300	1.00	1.00	1.00	1.50	1.50
Standard 5	1 g Standard 3 + 9 g H_2O	0.0400	0.0600	0.200	0.200	0.200	0.300	0.300
Standard 6	1 g Standard 3 + 29 g H_2O	0.0133	0.0200	0.0667	0.0667	0.0667	0.100	0.100

To prepare the Standard 1, weigh ~5 g of the Dionex Seven Anion Standard on an analytical balance to the nearest 0.0001 g and add ~5 g of ASTM Type I water for a 1:2 dilution. Use Standard 1 to prepare Standards 2 & 3. Use Standard 3 to prepare Standards 4, 5, & 6. Carefully record all of the masses when preparing calibration standards and calculate the true concentrations to three significant figures. Transfer the standards to pre-cleaned (SOP 2) and labeled plastic 150-mL bottles.

10.0 OPERATION OF DIONEX ION CHROMATOGRAPH

Table 2. Ion Chromatography Parameters for the Dionex ICS-1500

IC Parameter	Instrument Settings
Flow Rate (mL/min)	1.2
Injection volume (μL)	10 or 25 (check sample loop)
Column Temperature (°C)	30
Cell Temperature (°C)	35
Suppressor current, mA	31
Elution order	F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-}, and SO_4^{2}

10.1 Before starting the instrument, check the logbook to see if any problems have occurred. Enter this use into the logbook.
10.2 Prepare fresh eluent and place it in the eluent reservoir. Replace the eluent reservoir and insert the draw tube until it reaches the bottom of the bottle.
10.3 Check the wastewater reservoir. Dump the wastewater into the sink if it reaches about half full.
10.4 Verify the Dionex IC1600 instrument is turned on with power indicator lit green. Verify the Dionex AS-DV Automated Sampler is turned on by the connected indicator lit green. If not the main power switches are located on the back sides of the instruments.
10.5 Double click on the <**Chromeleon 7>** icon located on the desktop.
10.6 Click the **<Instruments>** tab at the bottom left corner.
10.7 Click the <Pump_ECD> tab at the top of the window to view the replicate IC panel on the screen.
10.8 Verify the instrument is connected to the *software* by making sure the green light is lit on the screen. Verify the autosampler is connected by clicking the <Sampler> tab and the Connected indicator is lit with a green light on screen.
10.9 On the <Pump_ECD> tab, locate the <Pump> control box drag the eluent fill line to 2. Set the flow rate to 1.2 mL/min and click <ON> to start the pump.
10.10 Locate the <Supressor> control box and change TYPE to AERS_4mm, change CURRENT to 31 mA and drag icon to the left to turn the suppressor ON.
10.11 Click <Command> on the top tool bar. Set the Cell Temperature.Norminal to 35.0ºC.

10.12 Locate the <Column Oven> control box and set the column heater temperature to 30.0 °C.
10.13 On the top tool bar click <Monitor Baseline> then <OK>.
10.14 Check the flow from the suppressor to be sure eluent is flowing through the system. You should see a regular pattern of bubbles and eluent passing through the tubing to the waste container. If bubbles aren't present, increase current (in Suppressor control box) to about 50 mA until bubbles are present then set it back to 31 mA.
10.15 Allow the system to equilibrate for at least 30 minutes.
10.16 While Monitoring Baseline, stretch the output signal as far as possible to check for a sine wave pattern (The screen should show the ECD_1 detector reading directly). This indicates that there are bubbles in the eluent line. To reduce the sine wave patters, loosen the knob on the left pump (the priming pump) for a couple of seconds and observe is bubbles are released. Close the valve and continue to monitor the signal until the background signal is as flat as possible. This is a good time to also fill the sample vials and load the autosampler.

10.17 **Preparation of samples and loading of autosampler vials into racks.**

10.17.1 Allow samples to come to room temperature to avoid formation of air bubbles in vials.
10.17.2 Use gloves when handling vials. Autosampler vials should be labeled according to the sample container from which they were removed.
10.17.3 After rinsing, place the empty vials into an autosampler cassette. If you are doing trace-level analyses, use forceps to handle the vials and avoid touching any surface that will be wetted with sample.
10.17.4 Load each standard/sample into a sample vial in the following sequence: 2 LRBs, Calibration Standards (lowest to highest concentration), 2 LRBs, QCS, undiluted samples, Calibration Check Standard (CCS of intermediate concentration), LRB, diluted samples, seven replicates of Calibration Standard 5 (to determine the MDL), and one End Calibration Check Standard (ECCS of a lower concentration) followed by a 2 LRBs.
10.17.5 Vials will be filled using a disposable 10-mL BD syringe, to which a disposable 0.2-μm syringe filter has been attached. First, draw a few milliliters of sample/blank/standard into the syringe and discard. Then, draw another few milliliters of sample into the syringe, attach a filter and depress the syringe plunger to discharge about 2 mL of the sample into a waste container. This rinses the filter. Fill the autosampler vial to the fill line marked on the vial body. After filling, inspect the vials to make sure no air bubbles are trapped at the bottom.

10.17.6 Inspect each cap for damage (nicks, scratches, etc.). Refer to the Appendix of this document for the correct configuration and install the caps in the vials. Use forceps when handling the caps to prevent contamination.

10.17.7 An insertion tool (P/N 037987) ensures that the cap is inserted to the proper depth. The flat end of the tool inserts the cap to the proper depth for a sample (i.e., the top of the cap is flush with the lip of the vial).

10.17.8 After pushing the cap into the vial, shake off any liquid that has been forced into the cap socket. Do not use laboratory wipes to blot liquid from the cap sockets; wipes leave fibers, which can accumulate in the liquid flow path and cause increased backpressure.

10.17.9 On the <Sampler> tab you can use the *Commands* box Raise the Needle and to move carousel as you load your samples.

10.17.10 Load the vials in the autosampler carousel, with adapters. Notate each sample with placement number on carousel as this indicates the order in which the vials are sampled.

10.17.11 Make sure vials are loaded in the correct sequence.

10.17.12 Under the <Settings> command box change *Deliver Volume* to 250 and move the *Vial Position* to 1.

10.18 **Setting up a Sequence**

10.18.1 Click “Create” on the top toolbar, followed by “Sequence” to activate a new sequence.

10.18.2 A screen will appear asking for information about your sequence. Input the total number of vials, injections per vial (2), start position of your first vial (should be 1), and injection volume to 250.

10.18.3 Select “Next” for Methods and Reporting.

10.18.4 For “Instrument Method” click <Browse>, then <ENVIRO>, then <Open>.

10.18.5 For “Processing Method” click <Browse>, then <New Processing Method>, then <Open>.

10.18.6 Select <Next>, then <Finish> to generate the sequence. Name the sequence by entering “GlenHelenMMDDYYYY” in object name and click <Save>.

10.18.7 The new sequence will pop open and here you can edit the names of standards, blanks, and samples to reflect what is at each location in the sampler carousel and click <Save>.

10.18.8 Save the edited file by clicking save in the top window and open an old anion sequence file from the browser menu on the left hand side of the screen. Select “Shutdown Anion.pgm”. Right click and select copy to copy this program and add it to the end of your sequence.

10.18.9 Reopen the sequence just created. Paste the "Shutdown Anion.pgm" into the sequence just under the new sequence where the other .pgm files are located. This will shut down the IC automatically at the end of the analysis.

10.18.10 Append a additional entries to the end of the sequence by selecting the last entry and simultaneously pressing the control and down keys. Make two "blank" entries. On the instrument column for the last vial change method from ENVIRO to Shutdown.

10.18.11 Save the final version of the sequence file.

10.2 Check the "conductivity" reading to be sure it is stable. When the IC is stable, record the total conductivity and total backpressure and other information on the logbook.

10.19 Return to the main menu by clicking on the "Instrument" tab at the bottom left corner. Select "Pump_ECD"

10.20 Click on the "Stop" on the top tool bar to end the real time data acquisition.

10.21 Return to the desired sequence file by selecting "Data" in the bottom left corner. Select you named sequence. Click "Start" to begin analysis.

10.3 After analysis has finished, must print the Method and export the data.
Exporting IC Data:
Click one of your finished samples, click report designer, select "Anion", click Chromeleon at the top right corner, click Export, Select Current sequence, then ok.
Print/Export IC Method
If printing is not possible, copy method and paste onto an appropriate text or Word document and then save on USB.

11. DATA ANALYSIS

Since the elution order of the seven analyzed anions is known, a set of six standards will be run on the IC (Dionex) in order to determine the anions' retention time. The established retention time for the anions will be used to assess the identities of the anions detected in the water samples. In addition, a calibration curve for each anion will be generated based upon how the instrument response (analytical signal) changes with the concentration of the analyte from the lowest to highest concentrated standard.

A calibration curve is an analytical method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration. Calibration curves will be generated on Excel or another comparable data analysis software (e.g., Origin or SigmaPlot) by plotting the analytical signal (analyte peak area) of the instrument against the concentration of the analyte (anion).

A sufficient calibration curves should display a linear trend with a linear regression coefficient (R^2) of at least 0.99. The linear regression equation for the plot will be obtained from the data analysis software and will be used to compute the anion concentrations in all unknown samples. The analytical signal (µS*Min) and retention time of each anion will be obtained from chromatograms generated by the IC Chromeleon software.

The six standards were prepared using a serial dilution method, so dilution factors must be taken into account to accurately compute the concentration of each standard analyte. The following equation is used to accurately calculate anion concentration:

$$[\text{anion concentration}] = (\text{original anion conc.})(\text{diltuion factor})$$

where

the original anion concentration is obtained from the original stock solution or original standard used to prepare the given standard (Table 1), and the dilution factor is the ratio of final volume/aliquot volume (final volume = aliquot + diluents). In this case, the volume is considered to be equivalent to the mass of the solution (Table 1).

It is important to note, if any anion is detected at concentrations above the highest standard (Std 1), those samples must be appropriately diluted in order to determine the anion concentration.

The analyte peak areas (µS*Min) or concentration from standard 6 (most diluted standard) define the limit of detection (LOD) for each anion. Levels of anions below their respective LODs do not mean the anions were completely absent from that particular site. It only indicates the level of analyte cannot be detected within an acceptable confidence limit. Greater uncertainty is associated with the integration of peaks with lower intensities, so it may be necessary to redraw or reselect the base line of anion peaks for more accurate analyte peak areas (µS*Min).

LFM Calculations
If the fortified concentration is less than the observed background concentration of the unfortified matrix, the recovery should not be calculated. This is due to the difficulty in calculating accurate recoveries of the fortified concentration when the native sample concentration is so high.

Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample. Percent recovery should be calculated using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$

where, R = percent recovery.
C_s = fortified sample concentration
C = sample background concentration
s = concentration equivalent of analyte added to sample.

Until sufficient data becomes available (usually a minimum of 20 to 30 analysis), assess laboratory performance against recovery limits of 75 to 125%.

12. REFERENCES

EPA Method 300.1, Determination of inorganic anions in drinking water by ion chromatography, Revision 1.0.

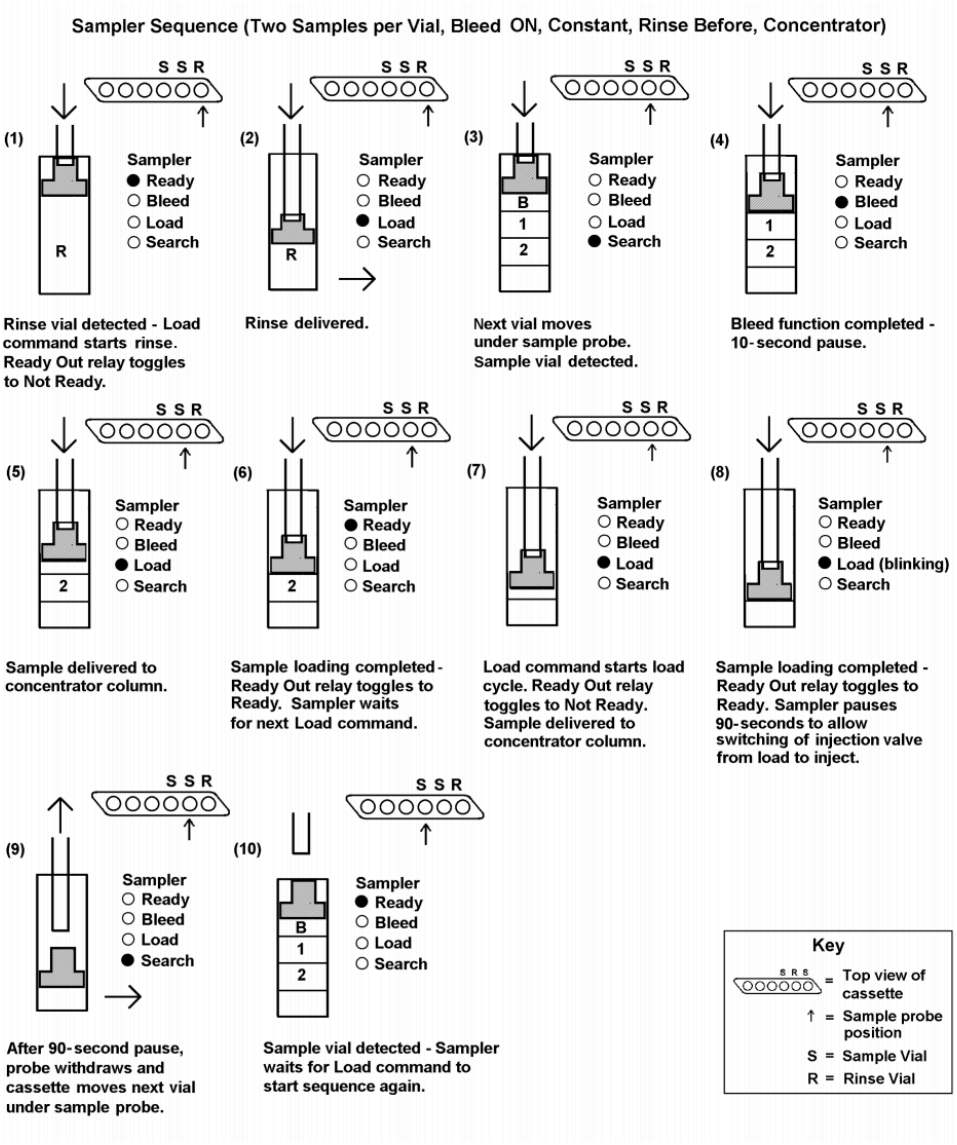
Dionex IC1500 and AS40 operation manuals.

Standard Operating Procedure 2. Cleaning sample containers for anion analysis.

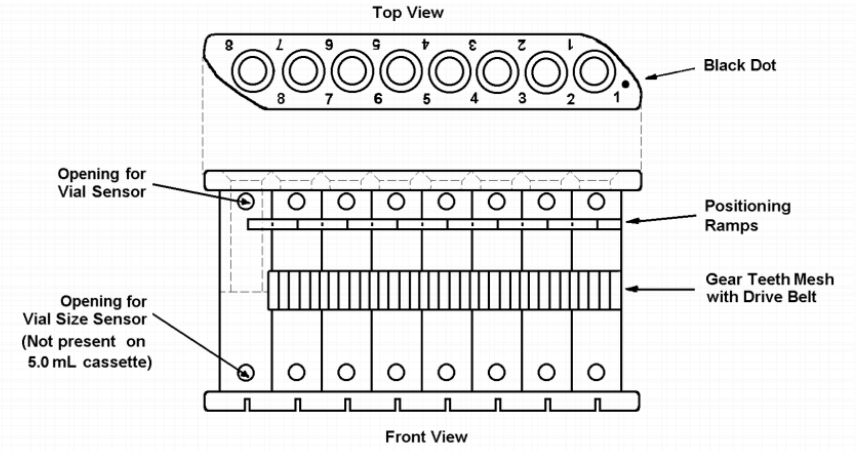
Standard Operating Procedure 3. Sampling at Glen Helen Nature Preserve.

Appendix

Autosampler Run Mode



Sample Cassette



STANDARD OPERATING PROCEDURE 5.7

FOR INDUCTIVELY-COUPLED PLASMA – OPTICAL EMISSION SPECTROMETRY (ICP-OES) ANALYSIS OF WATER AND SEDIMENT SAMPLES

July 5, 2018

By

Audrey E. McGowin, PhD

1.0 SCOPE AND APPLICATION

This method utilizes inductively coupled plasma – atomic emission spectrometry (ICP - AES) to determine selected trace elements found in water and sediment samples. Prepared samples are poured into an autosampler tube and numerically placed into an autosampler rack. The autosampler draws up a selected amount of sample and introduces it into the instrument. A peristaltic pump then draws the sample into the nebulizer where it aerosolized with argon gas. The aerosolized sample is ionized by inductively coupled plasma. Each element emits a characteristic wavelength that is used for detection. **Table 1** includes, but is not limited to, elements that may be analyzed by ICP.

Table 1. Analytes with preferred wavelengths.

Analyte	Symbol	CAS No.	Wavelength (nm)
Aluminum	Al	7429-90-5	308.215
Arsenic	As	7440-38-2	188.980 193.691 (axial)
Cadmium	Cd	7440-43-9	228.802 226.499 (axial)
Chromium	Cr	7440-47-3	276.653 205.557 (axial)
Copper	Cu	7440-50-8	324.747
Iron	Fe	7439-89-6	38.204, 259.933
Manganese	Mn	7439-96-5	93.305, 257.604
Nickel	Ni	7440-02-0	231.602 (axial)
Phosphorus	P	7723-14-0 (red) 2185-10-3 (white)	213.618, 253.5, 214.912 (axial)
Lead	Pb	7439-92-1	220.350 (axial)
Strontium	Sr	7440-24-6	421.534, 460.733
Zinc	Zn	7440-66-6	206.200 213.855 (axial)

2.0 SUMMARY OF METHOD

This method applies to sample analysis by ICP-OES for trace metals and phosphorus in water and digested sediments.

3.0 HEALTH AND SAFETY

Personal protective equipment (PPE) should be worn at all times while in the lab. This includes lab coat, nitrile gloves, and safety glasses at a minimum in addition to long pants and closed toes shoes. Nitric and hydrochloric acids are used widely as a diluent for ICP analysis and are very acidic and dangerous. Any handling of acids should be performed in the fume hood. It is important to remember that when mixing acid and water, acid should always be added to water. If eye or skin contact occurs, flush with copious amounts of water. Immediately report any spills to appropriate personnel for proper cleanup. Unused acids should be neutralized in the hood and additional hazardous waste should be disposed of properly.

ALWAYS pipette straight up and down and NEVER pipette directly from the metal analyte containers. Metal analyte should always be poured into clean beakers for pipetting. Pipettes should be allowed to drain for a minimum of 20 seconds into the appropriately labeled volumetric flasks and touch-dropped. Metal analyte containers should never be open for longer than necessary and preserved in sealed zip-loc bags when not being used. ALWAYS use a clean pipette for each analyte and rinse pipettes after use.

4.0 EQUIPMENT AND SUPPLIES

Varian ICP with computer control and high purity grade (99.99%) argon gas supply
Autosampler
Autosampler tubes
1-L Class A Volumetric flask (1)
500-mL Class A Volumetric flask (1)
100-mL Class A Volumetric Flasks (7)
50-mL TD Pipette (2)
25-mL TD Pipette (2)
10-mL TD Pipette
5-mL TD Pipette
1-mL TD Pipette (1)
Micropipettor with trace metal grade tips (1)
50-mL Beakers (7)
All glassware should be cleaned in accordance with SOP 1

5.0 SAMPLE HANDLING AND PRESERVATION

Sample collection and preparation should be performed in accordance with SOPs 1, 3, 4, or 14 (for phosphorus). Samples will be in good condition for ICP analysis for up to 6 months, but it is preferred that ICP analysis be done within a month or less.

6.0 REAGENTS AND STANDARDS

Trace metal grade nitric acid, concentrated (sp. Gr. 1.41)
Reagent grade water, ASTM Type I
Standard Stock Solutions (1000 ppm in 4% nitric or hydrochloric acid) made from ultra-high purity grade chemicals for each element analyzed.

6.1 A Mixed Standard Solution is prepared according to Table 2 below.

Table 2. Preparation of Mixed Standard Solutions for calibration.

Element	Concentration of primary standard (mg/L)	Volume of primary standard diluted to 500 mL with 4% HNO_3	Final concentration Mixed Std (mg/L)
Al	1000	5.00	10.00
As	1000	5.00	10.00
Cd	1000	5.00	10.00
Cr	1000	5.00	10.00
Cu	1000	5.00	10.00
Fe	1000	5.00	10.00
Mn	1000	5.00	10.00
Ni	1000	5.00	10.00
P	1000	5.00	10.00
Pb	1000	5.00	10.00
Sr	1000	5.00	10.00
Zn	1000	5.00	10.00

Calibrations Standards 1-5 are prepared by diluting the specified volume of the Mixed Standard to the mark with 4% nitric acid (or the same acids used in sample preparation and/or digestion) in labeled 100-mL volumetric flasks as is described in Table 3.

For samples with high calcium and sodium, a matrix modifier may need to be added to the standards to match the matrix of the samples.

Table 3. Preparation of Calibration Standard Solutions with Final Concentrations.

mL Mixed Std diluted to 100 mL	0.000	0.200	0.500	1.00	5.00	10.00	50.00
Element	**Blank (ppm)**	**STD 1 (ppm)**	**STD 2 (ppm)**	**STD 3 (ppm)**	**STD 4 (ppm)**	**STD 5 (ppm)**	**STD 6 (ppm)**
Al	0.000	0.020	0.050	0.100	0.500	1.000	5.000
As	0.000	0.020	0.050	0.100	0.500	1.000	5.000
Cd	0.000	0.020	0.050	0.100	0.500	1.000	5.000
Cr	0.000	0.020	0.050	0.100	0.500	1.000	5.000
Cu	0.000	0.020	0.050	0.100	0.500	1.000	5.000
Fe	0.000	0.020	0.050	0.100	0.500	1.000	5.000
Mn	0.000	0.020	0.050	0.100	0.500	1.000	5.000
Ni	0.000	0.020	0.050	0.100	0.500	1.000	5.000
P	0.000	0.020	0.050	0.100	0.500	1.000	5.000
Pb	0.000	0.020	0.050	0.100	0.500	1.000	5.000
Sr	0.000	0.020	0.050	0.100	0.500	1.000	5.000
Zn	0.000	0.020	0.050	0.100	0.500	1.000	5.000
mL Mixed Std diluted to 100 mL	0.000	0.200	0.500	1.00	5.00	10.00	50.00

Be sure standards are mixed thoroughly. Standards and samples are poured into labeled autosampler tubes for ICP analysis. The tables for ICP calibration standard preparation should be consulted for data entry into the computer.

6.2 Blanks

Calibration Blank or Reagent Water Blank (CB) – The calibration blank used in establishing the analytical calibration curve for aqueous samples and extracts is prepared by acidifying reagent water to the same concentrations of the acids as used for the standards.

Laboratory Reagent Blank (LRB) – The LRB must contain all the reagents in the same volumes as used in the processing of the samples. The LRB must be carried through the same entire preparation scheme as the samples including sample digestion, when applicable.

6.3 Quality Control Sample (QCS) is used to periodically verify calibration standards and to verify instrument performance. It is obtained from an outside source different from the stock solutions used in preparing calibration standards. The concentration of the analytes should be ≥1 mg/L.

7.0 QUALITY CONTROL

7.1 Initial Demonstration of Performance

7.1.1 The linear dynamic range (LDR) must be established for each wavelength used. Sample analyte concentrations that are >90% of the determined upper LDR limit must be diluted and reanalyzed.

7.1.2 Results of the analysis of quality control samples (QCS) must be within ±5% of the stated values. If not, the source of the problem must be identified and corrected before preceeding.

7.1.3 Method Detection Limit (MDL) – MDLs are determined but all wavelengths utilized, analyzing the reagent water blank (blank) that has been fortified to a concentration that is two to three times the estimated instrument detection limit (IDL). For this analysis, the lowest standard solution can be used to determine the MDL. Analyze seven aliquots of this solution that has been through the entire analytical process (filtering, dilutions, calculations, etc.). Calculate the MDL using the following equation:

MDL = (t) x (S)

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates].
S = standard deviation of the replicate analyses.

MDLs must be sufficient to detect analytes at the required levels of compliance monitoring regulation.

7.2 A laboratory reagent blank must be analyzed with each batch of 20 or fewer samples of the same matrix. LRB values that exceed the MDL indicate possible contamination. A standard should also be analyzed every 20 samples ensure there is no instrument drift.

8.0 PROCEDURE

8.1 PROCEDURE FOR ICP-AES MACHINE OPERATION

The steps listed below are in the order that they need to be performed to ensure the most stable set up. Not following this order can cause the computer system to not communicate with some of the equipment in an appropriate manner.

8.1.1 Turn on the autosampler. This must be done before the software is opened or it will not recognize that the autosampler is present. The switch is on the back. The auto sampler is on when the green light is on the front.

Make sure that there are no blockages in the nebulizer. The use of a flashlight, to shine in and around the nebulizer, is necessary to ensure that the flow of gas into and out of the nebulizer is not blocked.

8.1.2 Replace the autosampler water. The water is located in front of the nebulizer in a flask. It must be filled with fresh high quality water. This water is used to rinse the auto sampler and must be free of as many contaminants as possible.

8.1.3 Open exhaust vent above the ICP machine.

8.1.4 Turn on the Argon gas. The valve is turned until it is completely open.

8.1.5 Turn on the water pump under ICP machine. Make sure the water is does not need to be changed. Check water pressure.

8.1.6 Make sure waste tubes are in the waste container.

8.1.7 Turn on and set up the computer. Open the program on the desktop titled " 710ES ICP". This program must be open for at least 20 min before running samples.

8.1.8 Click "worksheet" then "new".

8.1.9 Go to "Create a New Method" using the Quantitative Tab

8.1.10 Click DJ5890f1\Varian

8.1.11 Click VAIMDB Chemistry Department

8.1.12 Click on the McGowin\Chem Folder

8.1.13 Name the worksheet. The worksheet name should include the date the samples were taken and GLEN HELEN.

8.1.14 Lock tubing into place on the peristaltic pump. There are two tubes one tube that feeds to the nebulizer from the auto sampler and one that drains condensed liquid from the spray chamber. The tubes are stretched over the pump and locked into place with pressure bars.

8.1.15 Make sure the drain tubing is in the drain container.

8.1.16 Verify that the instruments are still on.

8.1.17 Click instrument set up then verify that the water cooler flow and gas flow are flowing properly.

8.1.18 Light torch. To light the torch, click the plasma on button in the tool bar. The instrument must come to thermal stability before calibration and

analysis. The torch must be allowed to operate for at least 30-60 minutes before any measurements are taken.

8.1.19 Click "Edit Method" to set up parameters and elements to be tested by clicking on them on the periodic table that shows up. Accept the recommended wavelength for each element.

8.1.20 Set up standards

8.1.21 Check analysis

8.1.22 Select 6 for the number of standards

8.1.23 Fill in concentrations for each element in the table for the standards.

8.1.24 Set the correlation coefficient to 0.95

8.1.25 Change read time to 10s

8.1.26 Close method editor and accept the warning that pops up.

8.1.27 Load Samples. The standards need to be loaded with the blank located at the front of the autosampler (the front being the side facing you). The samples should be loaded with the first sample in the back of the auto ampler or starting on the side closest to the ICP machine. Place plastic in between samples and standards for support.

8.1.28 Set up sequence.

8.1.29 Go to sequence editor. Allow for one blank before the standards.

8.1.30 Set the dilution factor to 1

8.1.31 Check the box for auto sampler, not manual. This is located to the right of the screen.

8.1.32 SAVE FILE.

8.1.32 Analysis. Click the analysis button to start analyzing. All samples to be analyzed should be highlighted Yellow.

8.2 Turning off the machine.

8.2.1 Once all samples are done analyzing and results are complete, the machine can be disconnected from the autosampler and argon gas. All data should be checked before this process is started. Also save all data and print before starting.

8.2.2 Turn off the torch.

8.2.3 Wait 10 minutes before turning of gas. The rest of this process can be continued in the mean time.

8.2.4 Release peristaltic pump pressure bars and tubing

8.2.5 Shut off water pump.

8.2.6 Turn off auto sampler.

8.2.7 Close exhaust vent.

8.3 Error trouble shooting

8.3.1 If the torch goes out. Click "OK" or "YES" when the error pops up. Wait a few seconds. Light the torch again.

8.3.2 IF errors occur in data, the percent error can be changed to a higher value. This is usually done when there is a lot of background noise or if large gaps are found between the peak intensity. This value can be changed by

changing the values in the method editor MulitCal area. Save the changes then return to view the data.

9.0 DATA ANALYSIS

Most of the data analysis will be performed by the computer. The standards prepared in section G.1 above will be used to aid with the data analysis. By plotting the intensity of the analyte signal versus the concentration of the standards a linear curve should form. The curve must be linear and have a correlation coefficient (R^2) value of greater than 0.99 in order for the curve to be valid. The equation of the line will be used to determine the concentration of the unknowns from the analyte intensity. All of this will be done with the ICP software.

This method will not work for unknowns that register higher analyte signal than the highest standard. These samples will have to be quantitatively diluted to the point at which analyte signal can fall in the linear range with the 4% nitric acid solution. The concentration determined from the software will then need to be scaled up to the undiluted value i.e. if the solution underwent a 1:1 dilution then the concentration from the computer would need to be multiplied by 2.

10.0 REFERENCE

U.S. Environmental Protection Agency. Method 200.7: Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry, revision 3.3, EPA 600 4-91/010 June 1991.

Acid Digestion for the Determination of Phosphorous in the Filtrate of a filtered Water sample by Inductively-Coupled Plasma – Atomic Emission Spectrometry (ICP-AES)

SOP 7.5

Prepared by: Baxter J. Foskuhl, Lee A. Raska, Clara Leedy

Revised: October 12th, 2019
A. E. McGowin, PhD

A. SCOPE AND APPLICATION

This procedure utilizes analytical chemistry methods to extract phosphorous in water samples from tributaries in Caesar Creek Lake, in Waynesville, Ohio. Water samples were collected, filtered, and acid digested in preparation for inductively coupled plasma (ICP) analysis.

B. SUMMARY OF METHOD

This method applies to water sample preparation for analysis by ICP-AES for phosphorous.

C. HEALTH AND SAFETY

Personal protective equipment (PPE) should be worn at all times while in the lab. This includes lab coat, nitrile gloves, and safety glasses at a minimum in addition to long pants and closed toes shoes. Nitric acid is used for digestion of the sediment samples and is very acidic and dangerous. Any handling of nitric acid should be performed in the fume hood. It is important to remember that when mixing acid and water, acid should always be added to water. If eye or skin contact occurs, flush with copious amounts of water. Immediately report any spills to appropriate personnel for proper cleanup. Unused nitric acid should be neutralized in the hood and additional hazardous waste should be disposed of properly.

D. MATERIALS

- Plastic weigh dishes for air-drying samples
- Analytical balance
- Drying oven set at 105 °C
- Trace metal grade concentrated nitric acid
- Trace metal grade concentrated hydrochloric acid
- 10-mL graduated cylinder
- Hot plate
- Two 250-mL Erlenmeyer flasks for each sample (duplicate analyses) plus one for the blank
- Watch glass for each flask
- Glass funnels
- Whatman No. 41 filter paper or equivalent
- Whatman Glass microfiber filters, 1.5 μm pore size
- 100-mL volumetric flask for each sample
- Gloves

- Plastic weigh dishes or weigh paper for weighing samples.
- Aluminum weigh boats for determination of moisture content
- 125 mL Erlenymeyer filtration flask
- Vacuum hose, vacuum line set up

E. SAMPLE HANDLING AND PRESERVATION

After sample collection in the appropriately cleaned and labeled containers with accordance to SOPs 2 and 3, samples should be stored in the hood until analysis work up.

F. QUALITY CONTROL

An ASTM Type I water blank was prepared using the same methods applied to the water samples. Recoveries will be reported. A quality control sample will be analyzed alongside samples to determine percent recovery.

G. PROCEDURE FOR PREPARING SAMPLES

Filtration

1. Label all aluminum weigh pans. Dry both filter and aluminum weigh pans in drying oven at 105 °C for 48 hours.
2. Weigh filter and aluminum weigh pan. Record weights in notebook.
3. Assemble two separate vacuum filtration set ups for each type of filter. Have rinse bottles of HNO_3 and ASTM Type I H_2O nearby for in-between samples.
4. Filter an aliquot of sample through each set up, using a graduated cylinder to measure the volume of an aliquot of a sample. Record the volume used for each batch.
    a. Make sure the specifications of the graduated cylinder used are in laboratory notebook.
5. Vacuum filters for ~ 60 seconds.
6. Place filter samples into corresponding weigh pan, place in oven at 105 °C for 48 hours.
7. Filtrate water samples can be then transferred to a pre-labeled bottle pre-

cleaned according to SOP 2.

a. Make sure container is not glass, and at least 50 mL in volume.

8. After in oven, transfer samples to a desiccator under vacuum for 24-48 hours. After the time, make sure to turn of the vacuum and slowly release the pressure from the desiccator.
9. Weigh samples again, recording weights in notebook. The samples are now ready for the acid digestion step.

Acid Digestion

10. IN THE HOOD, add 2.5 mL conc. HNO_3 and 10 mL conc. HCl to each sample and swirl to create a slurry. Cover with a watch glass and place flasks on a hot plate.
11. Heat samples at 95 ± 5 °C for 15 minutes without boiling. Use a beaker of water with a thermometer to establish temperature on the hot plate.
12. Allow the sample to cool before diluting to volume in a 50-mL volumetric flask with Type I reagent water.
13. If a precipitate forms upon cooling, add 10 mL of conc. HCl. Do not do this if there is no precipitate. The precipitate should dissolve. Dilute to 50 mL with reagent grade water.
14. Mix the sample well and transfer this sample to a pre-cleaned (SOP 2) plastic bottle. This is the sample that will be analyzed by ICP-AES according to SOP 5.

H. DATA ANALYSIS

Refer to SOP 5.7 for ICP analysis. Results will be reported as the mean of duplicate analyses for each metal for each sample. A mean and standard deviation for the recovery of phosphorous in the SRM will be reported. Samples will be corrected for moisture content.

I. REFERENCES

1. Chunlong Zhang, Fundamentals of Environmental Sampling and Analysis, Wiley-Interscience, 2007, p. 380.

2. Acid Digestion of Sediments, Sludges, and Soils, USEPA Method 3050B, 1996.

Acid Digestion for the Determination of Phosphorous on Filters from filtered water samples by Inductively-Coupled Plasma – Atomic Emission Spectrometry (ICP-AES)

SOP 7.4

Prepared by: Baxter J. Foskuhl, Lee A. Raska, Clara Leedy

Revised: October 12th, 2019
A. E. McGowin, PhD

A. SCOPE AND APPLICATION

This procedure utilizes analytical chemistry methods to extract phosphorous in water samples from tributaries in Caesar Creek Lake, in Waynesville, Ohio. Water samples were collected, filtered, and acid digested in preparation for inductively coupled plasma (ICP) analysis.

B. SUMMARY OF METHOD

This method applies to water sample preparation for analysis by ICP-AES for phosphorous.

C. HEALTH AND SAFETY

Personal protective equipment (PPE) should be worn at all times while in the lab. This includes lab coat, nitrile gloves, and safety glasses at a minimum in addition to long pants and closed toes shoes. Nitric acid is used for digestion of the environmental samples and is very acidic and dangerous. Any handling of nitric acid should be performed in the fume hood. It is important to remember that when mixing acid and water, acid should always be added to water. If eye or skin contact occurs, flush with copious amounts of water. Immediately report any spills to appropriate personnel for proper cleanup. Unused nitric acid should be neutralized in the hood and additional hazardous waste should be disposed of properly.

D. MATERIALS

- Analytical balance
- Drying oven set at 105 °C
- Trace metal grade concentrated nitric acid
- Trace metal grade concentrated hydrochloric acid
- 10-mL graduated cylinder
- 250 mL graduated cylinder
- 1000 mL graduated cylinder
- Hot plate
- Two 250-mL Erlenmeyer flasks for each sample
- Watch glass for each flask
- Glass funnels
- Whatman No. 41 filter paper or equivalent
- Whatman Glass microfiber filters, 1.5 μm pore size, 47 mm diameter
- 100-mL volumetric flask for each sample
- Gloves

- Aluminum weigh boats for weighing
- 1000 mL Erlenymeyer filtration flask
- Vacuum hose, vacuum line set up

E. SAMPLE HANDLING AND PRESERVATION

After sample collection in the appropriately cleaned and labeled containers with accordance to SOPs 2 and 3, samples should be stored in the hood until analysis work up.

F. QUALITY CONTROL

An ASTM Type I water blank was prepared using the same methods applied to the water samples. Recoveries will be reported.

G. PROCEDURE FOR PREPARING SAMPLES

Filtration

15. Label all aluminum weigh pans. Dry both filter and aluminum weigh pans in drying oven at 105 °C for 48 hours.
16. Weigh filter and aluminum weigh pan. Record weights in notebook.
17. Assemble two separate vacuum filtration set ups for each type of filter. Have rinse bottles of HNO_3 and ASTM Type I H_2O nearby for in-between samples.
18. Filter an aliquot of sample through each set up, using a graduated cylinder to measure out a given volume of sample. Record the volume used for each batch.
    a. Make sure the specifications of the graduated cylinder used are in laboratory notebook.
19. Vacuum filters for ~ 60 seconds.
20. Place filter samples into corresponding weigh pan, place in oven at 105 °C for 48 hours.
21. Filtrate water samples can be then transferred to a pre-labeled bottle pre-cleaned according to SOP 2.
    a. Make sure container is not glass, and at least 50 mL in volume.

22. After in oven, transfer samples to a desiccator under vacuum for 24 - 48 hours. After the time, make sure to turn of the vacuum and slowly release the pressure from the desiccator.
23. Weigh samples again, recording weights in notebook. The samples are now ready for the acid digestion step.

Acid Digestion

24. IN THE HOOD, add 2.5 mL conc. HNO_3 and 10 mL conc. HCl to each sample and swirl to create a slurry. Cover with a watch glass and place flasks on a hot plate.
25. Heat samples at 95 ± 5 °C for 15 minutes without boiling. Use a beaker of water with a thermometer to establish temperature on the hot plate.
26. Allow samples to cool, then filter the digestate through a Whatman No. 41 filter into a 50-mL volumetric flask.
27. Take the filter out of the funnel and place it back into the Erlenmeyer flask digestion vessel. Add 5 mL conc. HCl and heat on the hotplate at (95 °C ± 5 °C) to dissolve the paper. Remove flask from heat source and wash the inside and watch glass cover with Type I water.
28. Filter again with a Whatman No. 41 filter, and collect the filtrate in the same 50-mL volumetric flask.
29. Allow the sample to cool before diluting to volume with Type I reagent water.
30. If a precipitate forms upon cooling, add 10 mL of conc. HCl. Do not do this if there is no precipitate. The precipitate should dissolve. Dilute to 50 mL with reagent grade water.
31. Mix the sample well and transfer this sample to a pre-cleaned (SOP 2) plastic bottle. This is the sample that will be analyzed by ICP-AES according to SOP 5.7.
32. Report phosphorous results in mg/kg (dry weight).

a. The following equation (1) is used to determine the dry weight concentration of phosphorous on the filter:

$$(1)\ d.w. = \frac{c_x(\frac{mg}{L})\cdot 100\cdot \%\ Moisture}{m_{sed}\ (g)}$$

H. DATA ANALYSIS

Refer to SOP 5.7 for ICP analysis. Results will be reported as the mean of duplicate analyses for each metal for each sample. A mean and standard deviation for the recovery of phosphorous in the SRM will be reported. Samples will be corrected for moisture content.

I. REFERENCES

3. Chunlong Zhang, Fundamentals of Environmental Sampling and Analysis, Wiley-Interscience, 2007, p. 380.

4. Acid Digestion of Sediments, Sludges, and Soils, USEPA Method 3050B, 1996.

STANDARD OPERATING PROCEDURE 13.0

CALIBRATING A YSI PRO PLUS MULTIMETER
FOR pH, CONDUCTIVITY, AMMONIUM AND DO
AND OBTAINING FIELD MEASUREMENTS

September 30, 2013

By
Shannon Hennelly, Anna Foote, Megan Huddleson,
Renata Mitton and Abraham Kemboi

APPROVED:

_____

Audrey McGowin, Ph.D

1. Scope and Application

The YSI *Pro Plus* meter is a remote sampling meter used to acquire water-monitoring data instantly at a remote sampling site. Coupled with a *Quatro* cable the YSI meter can measure four parameters simultaneously. This method explains how to properly calibrate the four external sensors used in the sampling of the Glen Helen Nature Preserve: pH, DO, conductivity and ammonium. Each sensor must be correctly calibrated before being employed during field sampling.

This method also explains the correct sampling technique and the proper logging of field data both with the YSI multimeter and student notebooks.

2. Summary of Method

This method explains calibration of the YSI multimeter and sampling protocols.

3. Health and Safety

All six standards used have NFPA Codes of zero for health, reactivity, and flammability. Some of the pH standards may cause irritation to the eyes and skin. It is best to wear appropriate personal protective equipment (PPE) at all times while in the lab to avoid contact with the eyes and to avoid prolonged exposure to the skin. This includes lab coat, nitrile gloves, and safety glasses at a minimum in addition to long pants and closed toe shoes.

4. Equipment and Supplies

4.1. YSI Multimeter:
- 4.1.1. YSI *Pro Plus* Meter
- 4.1.2. YSI Quatro Cable
- 4.1.3. Four Sensor Probes (pH, DO, Conductivity, Ammonium)

4.2. YSI Storage Container (screw-on plastic cylinder)

4.3. YSI Field Cover (metal cover)

4.4. YSI Transport Container (grey rubber sleeve)

4.5. Craftsmen Carrying Case

4.6. Log Book

4.7. Student Notebooks

5. Reagents and Standards

5.1. Deionized Water (DI)

5.2. Conductivity:

5.2.1. YSI 3161 Conductivity Calibrator Solution (1000 µS/cm ± 0.50% at 25°C)

5.3. pH:

5.3.1. YSI 3821 Buffer Solution pH 4.00±0.01 at 25°C Lot# 13DIR

5.3.2. YSI 3822 Buffer Solution pH 7.00±0.01 at 25°C Lot# 13DIS

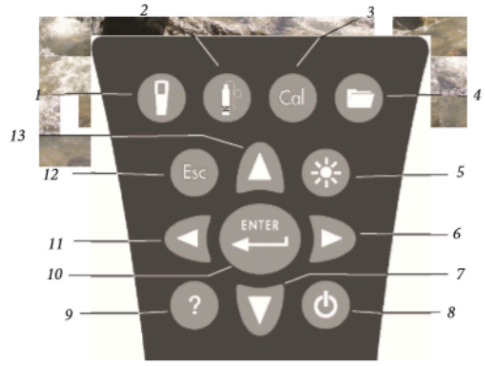
5.3.3. YSI 3823 Buffer Solution pH 10.00±0.01 at 25°C Lot#13DIT

5.4. Ammonium:

5.4.1. YSI 3841 1mg/L NH_4^+ -N Standard Lot# 13FID

5.4.2. YSI 3843 100mg/L NH_4^+-N Standard Lot#13FIF

KEYPAD



Number	*Key*	*Description*
1		**System** Opens System Menu from any screen. Use to adjust system settings.
2		**Sensor** Opens Sensor Menu from any screen. Use to enable sensors and display units.
3	Cal	**Calibrate** Opens Calibrate Menu from any screen. Use to calibrate all parameters except temperature.
4		**File** Opens File Menu from any screen. Use to view data and GLP files, set up site and folder lists, and delete data.
5		**Backlight** Press to turn the instrument backlight on and off and to adjust the display contrast when pressed with the left or right arrow key.

Number	Key	Description
6		**Right Arrow** Use to navigate right in alpha/numeric entry screens. Can be pressed simultaneously with Backlight key to increase display contrast.
7		**Down Arrow** Use to navigate through menus and to navigate down in alpha/numeric entry screens.
8		**Power** Press to turn the instrument on. Press and hold for 3 seconds to turn off.
9		**Help** Press to receive hints & tips during operation.
10		**Enter** Press to confirm selections, including alpha/numeric key selections.
11		**Left Arrow** Use to navigate left in alpha/numeric entry screens. Press to return to previous menu in all screens except alpha/numeric entry. Can be pressed simultaneously with Backlight key to decrease display contrast.
12		**Exit/Escape** Exits back to Run Screen. When in alpha/numeric entry screen, escapes to previous menu.
13		**Up Arrow** Use to navigate through menus and to navigate up in alpha/numeric entry screens.

6. Calibration Procedure

6.1. Dissolved Oxygen:

6.1.1. Insert the Quarto probe into a saturated storage container (make sure sponge is moist)

6.1.2. Push <Cal> to calibrate, select <DO>

6.1.3. Press <DO%>

6.1.4. Once % DO and temperature stabilize to slightly <100% press enter to "accept calibration".

6.1.5. Click <Cal> to finish.

Note: This is more of a *check* than an actual calibration.

6.2. Conductivity

6.2.1. Fill one beaker with high quality to use for washing.

6.2.2. Fill another beaker with enough conductivity solution (5.1.1) to be able to completely cover the conductivity probe (the conductivity probe is the black one with the metal prong extending out of the tip)

6.2.3. Remove the Quatro from the storage container and rinse with high quality water

6.2.4. Then gently shake dry.

6.2.5. Submerge completely in the conductivity stock standard for conductivity.

6.2.6. Press <CAL> for calibration, select “Conductivity”

6.2.7. Press the <Enter> button

6.2.8. Select specific conductance (“Sp. Conductance”) and press <Enter>.

6.2.9. Select “SPC-µs/cm” for the units.

6.2.10. Click <Enter> for calibration menu.

6.2.11. Once the meter readout stabilizes, press <Enter> to ”Accept Calibration”

6.2.12. Click <Enter>. Select User Field 1: Glen Helen.

6.2.13. After the probe calibrates rinse with DI water and store the probe in the clear plastic cylinder tube.

6.3. pH

6.3.1. The standards for pH (5.3) can be diluted 50:50 with high quality water. This is because they are buffer solutions which means they are resistant to pH change.

6.3.2. Make about 100 mL each in labeled and DI cleaned beakers.

6.3.3. Put high quality water in another beaker to use for washing.

6.3.4. Remove probe from container and rinse with high quality water and gently shake dry.

6.3.5. The pH probe is the gray one with the rounded glass electrode on the tip. Submerge it completely in the first pH stock solution (pH 4).

6.3.6. Press <CAL> for calibration, select “ISE2 pH” and press the <Enter> button.

6.3.7. Click <Enter> to show the calibration menu.

6.3.8. Once the meter readout stabilizes, press enter to "Accept calibration", click <Enter>.

6.3.9. The meter will then say "ready for point 2".

6.3.10. Rinse the probe and place into the next buffer (pH 7) and repeat the same procedure.

6.3.11. After stabilizing and pressing <Enter> the probe will ask for point 3.

6.3.12. Rinse and place the probe in the last buffer (pH 10). Again let the readout stabilize and press <Enter> to "accept calibration".

6.3.13. The probe will then ask for a fourth point, ignore this as only three are necessary.

6.3.14. Press <Cal> to finish and after the probe calibrates, rinse with water.

6.3.15. Store the probe in the clear plastic cylinder tube.

6.4. Ammonium

6.4.1. Pour about 50-100 mL of both ammonium standards (5.4) into two separated cleaned and labeled beakers.

6.4.2. Put high quality water in another beaker to use for washing.

6.4.3. Remove probe from container and rinse with high quality water and gently shake dry.

6.4.4. The ammonium probe is the gray one with the flat buttom. Submerge it completely in the first NH_4^+ solution (1 mg L^{-1})

6.4.5. Press <CAL> for calibration, select "ISE2 NH4" and press the <Enter> button.

6.4.6. Click <Enter> to show the calibration menu.

6.4.7. Once the meter readout stabilizes, press enter to "Accept calibration", click <Enter>.

6.4.8. The meter will then say "ready for point 2".

6.4.9. Rinse the probe and place into the next ammonium solution (100 mg L^{-1}) and repeat the same procedure.

6.4.10. After stabilizing and pressing <Enter> the probe will ask for point 3, ignore this as there are only two.

6.4.11. Press <Cal> to finish and after the probe calibrates, rinse with water.

6.4.12. Store the probe in the clear plastic cylinder tube.

6.5. After the multimeter is calibrated fill out the Log Book with today's date and sign it.

7. Preparing Probe for Field Sampling

7.1. Once probe is calibrated then it is ready to take out into the field.

7.2. Remove from storage container and switch to metal sampling cage.

7.3. Put about 5mL of DI water into the protective rubber sleeve

7.4. Slide the sleeve over the probe.

7.5. The probe will remain in the rubber sleeve just prior to sampling

8. Sample Collection and Logging Field Samples

8.1. Remove the rubber sleeve.

8.2. Gently submerge perpendicular to water flow (one person holds probe, one holds meter, all others write down the measurements as they are read aloud in their notebook/spreadsheet). Probe should now be submerged into our water.

8.3. Have the person holding the meter log real time readings from YSI read out.

8.4. Highlight "Log 1" sample, hit <Enter>, first go down to "folder", and press <Enter> and select your groups folder.

8.5. Next go down to "site" and press <Enter> and select the appropriate sampling site.

8.6. Lastly, select the top option ("Log Now") and press <Enter>. Logging has to be done while sampling is in progress.

8.7. To view logged data: press <Folder> and select "view data" work through the "site" directory and select the desired site data to be viewed and click <Enter>.

8.8. Next press "show data" and the data will be displayed in a tabulated format. The date will be on the left and the parameters with be on the top. Use the scroll arrows to display hidden data.

9. Exporting Data

9.1. Install

9.1.1. After sampling it is necessary to export the logged data to a computer.

9.1.2. This is done by using the YSI Pro Instrument software install CD. The first time the CD is put into a new computer the start-up window will ask to "install the driver". Do this first!

9.1.3. Once the driver has been installed the software maybe be downloaded and installed. Now the YSI meter maybe be connected to the communication cradle and the USB cable connected between the multimeter and the computer.

9.1.4. The meter will automatically turn and start the YSI software**.**

9.2. Export

9.2.1. Once the YSI multimeter is connected to a computer data may be retrieved.

9.2.2. Select "Retrieve Instrument Data" and click either "Select all" or "Date".

9.2.3. If date is chosen, you must input the sampling date to retrieve the correct data.

9.2.4. Next click the "Viewed Saved File/Data" tab.

9.2.5. In this menu data are prepared for display. The easiest option is display by "Site List".

9.2.6. You will also need to choose 6 parameter to display (no more may be selected so it is easiest to do this twice with six different parameters chosen both times).

9.2.7. In the new window the data are shown. The "edit" option in the top right allows for choosing different units. Once desired data are displayed select "Export" and check ".csv."

9.2.8. Choose an appropriate folder to save the data in and create a logical name and export.

9.2.9. The data can now be opened in excel.

9.2.10. From excel data, create tables for presentation and plot current data against archived data.

10. Reference

YSI *Professional Plus*. User Manual. **2009**

IX. APPENDIX C – Additional Figures and Plots

The following figure 1C is a plot of the fraction of NO_3^--[N] to DIN-[N] and the fraction of NH_4^+-[N] to DIN-[N] for each depth on each sampling date in Caesar Creek Lake for Year 2 from April 9th, 2019 to September 26th, 2019:

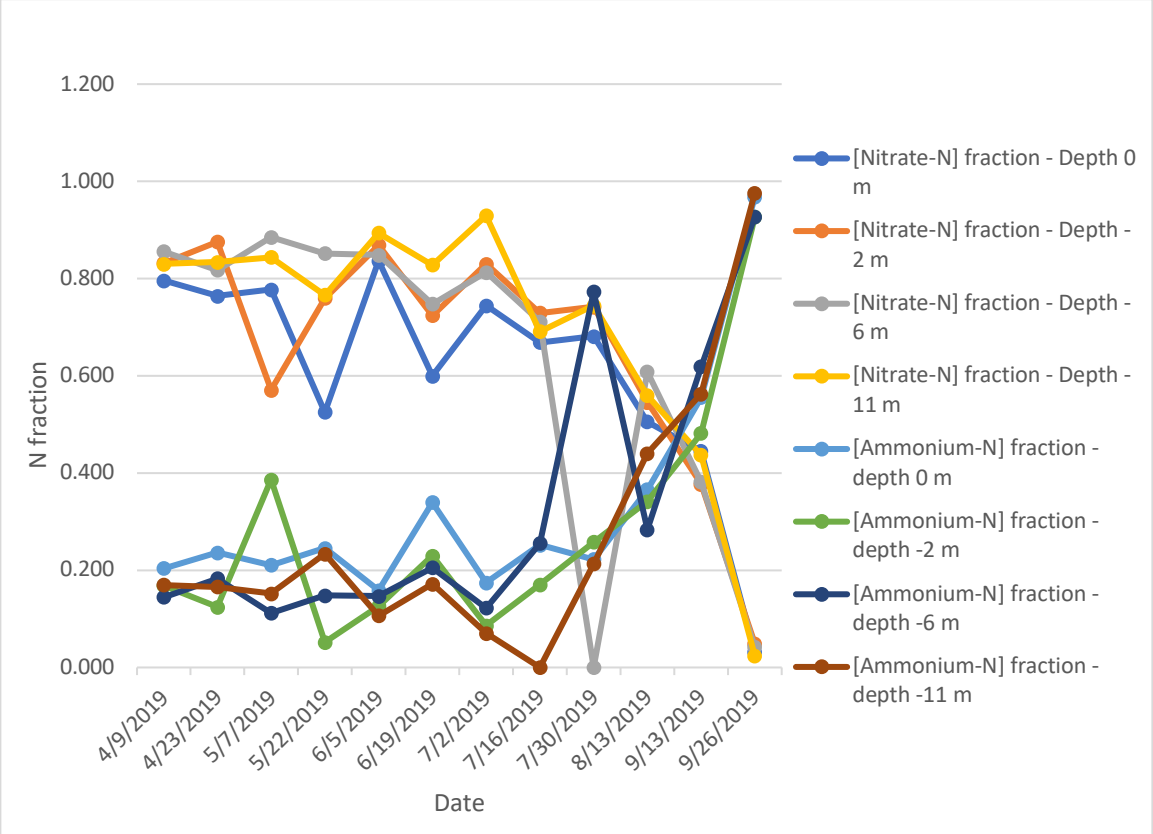


Fig. 1C - Plot of the fraction of NO_3^--[N] to DIN-[N] and the fraction of NH_4^+-[N] to DIN-[N] for each depth on each sampling date in Caesar Creek Lake for Year 2 from April 9th, 2019 to September 26th, 2019.

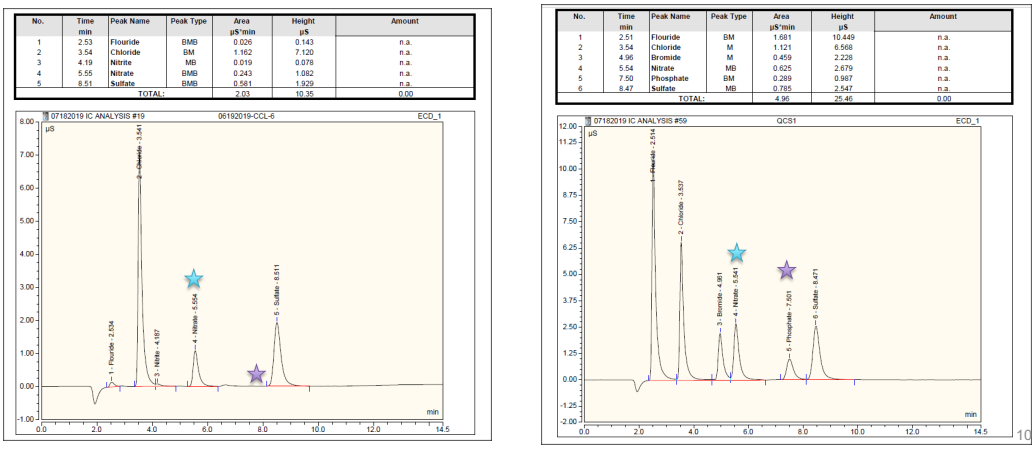
APPENDIX D - Selected Thesis Defense Presentation Slides

The following figures are selected slides from the defense of this thesis on December 12th, 2019.

The following figure 1D shows two chromatograms. On the left is an ion chromatogram from Caesar Creek Lake at a depth of 2 m on June 19th, 2019. On the right is a ion chromatogram of the quality control solution (QCS) used to for quality assurance. Nitrite was not able to be detected in the QCS due to its ease of being oxidized at room temperature. The chromatograms were obtained using conditions detailed in Table 2. The blue and purple stars denote where nitrate and phosphate elute with the system conditions. As seen, phosphate is not detected in the sample below and was not detected in the majority of samples analyzed.

WRIGHT STATE UNIVERSITY

Methods – Ion Chromatography - June 19th, 2019, CCL – depth -2 m



No.	Time min	Peak Name	Peak Type	Area µS*min	Height µS	Amount
1	2.53	Flouride	BMB	0.026	0.143	n.a.
2	3.54	Chloride	BM	1.162	7.120	n.a.
3	4.19	Nitrite	MB	0.019	0.078	n.a.
4	5.55	Nitrate	BMB	0.243	1.082	n.a.
5	8.51	Sulfate	BMB	0.581	1.929	n.a.
		TOTAL:		2.03	10.35	0.00

No.	Time min	Peak Name	Peak Type	Area µS*min	Height µS	Amount
1	2.51	Flouride	BM	1.681	10.449	n.a.
2	3.54	Chloride	M	1.121	6.568	n.a.
3	4.96	Bromide	M	0.459	2.228	n.a.
4	5.54	Nitrate	MB	0.625	2.679	n.a.
5	7.50	Phosphate	BM	0.289	0.987	n.a.
6	8.47	Sulfate	MB	0.785	2.547	n.a.
		TOTAL:		4.96	25.46	0.00

Fig. 1D - Two ion chromatograms showing a real sample from a depth of 2 m on June 19th, 2019, at Caesar Creek Lake (leftmost plot) and a quality control solution sample ((QCS), rightmost plot). The QCS sample had 10 mg/L of nitrate and phosphate. The blue and purple stars denote the nitrate and phosphate peaks, respectively. These chromatograms were obtained using operating conditions detailed in Table 2.

The following Fig. 2D shows the time logged YSI instrument data from May 7th, 2019 at -6 m at Caesar Creek Lake (upper plot), and Turkey Run (lower plot). DO, mg/L and ammonium-[N], mg/L concentrations were obtained in 1 second intervals for a period of two minutes. This is how all of the water quality data was obtained by the YSI instrument in this study. Ammonium-[N] tended to display a very stable response unlike dissolved oxygen, which required more time to stabilize in reading.

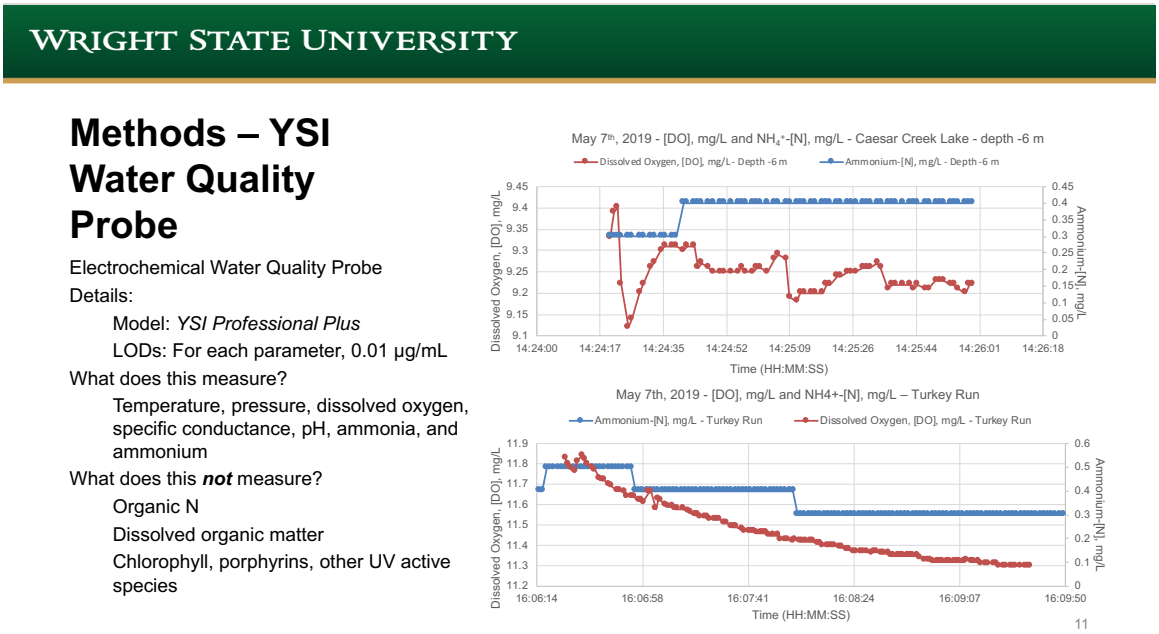


Fig. 2D - Logged YSI instrument data from May 7th, 2019 at -6 m at Caesar Creek Lake (upper plot), and Turkey Run (lower plot). DO, mg/L and ammonium-[N], mg/L concentrations were obtained in 1 second intervals for a period of two minutes.

The following figure 3D is a summary of the method development done to determine which filter would retain particulate phosphorous the most. Five trials were performed. For each trial, calibration curves ranging from 0.02000 mg/L to 10.00000 mg/L were created and analyzed using ICP-AES for phosphorous. The slopes and R^2 values of these calibration curves were plotted, and are shown in Fig. 3D. Additionally, both the glass microber and cellulose Whatman #1 filters were used in the analysis of Caesar Creek Lake and tributary samples for June 19th, 2019 and July 2nd, 2019.

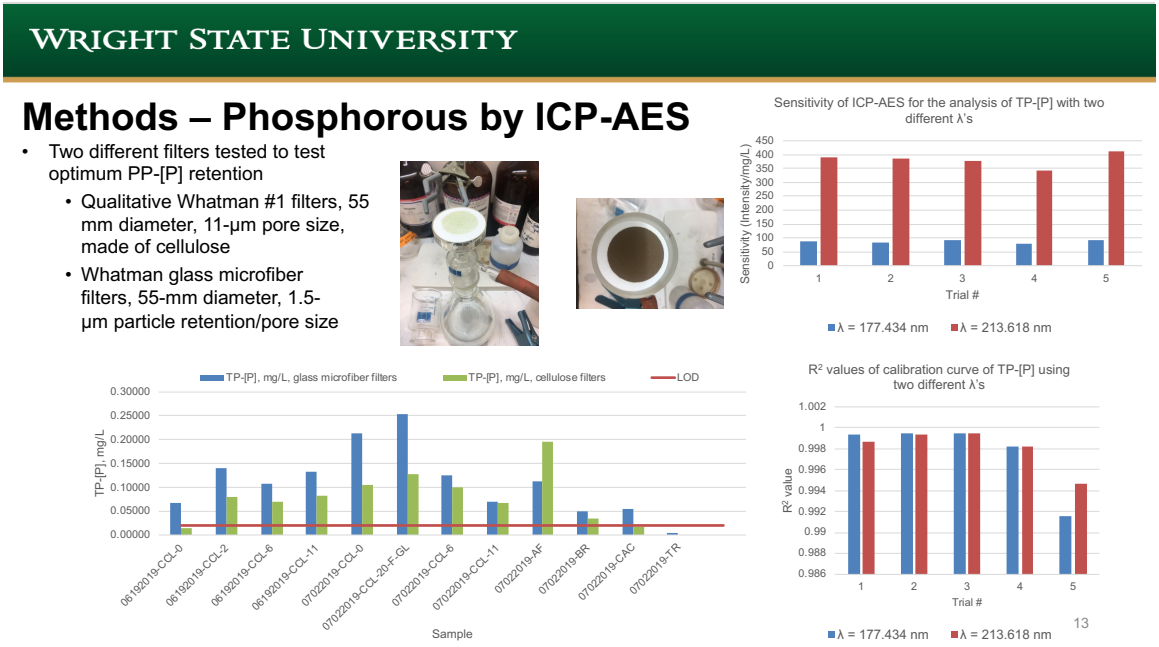


Fig. 3D - Summary of the method development done to determine optimum filter choice for the retention of particulate phosphorous. Five trials were performed. For each trial, calibration curves ranging from 0.02000 µg/L to 10.00000 mg/L were created and analyzed using ICP-AES for phosphorous. The slopes and R^2 values of these calibration curves were plotted, and are shown in Fig. 3D. Additionally, both the glass microfiber and cellulose Whatman #1 filters were used in the analysis of Caesar Creek Lake and tributary samples for June 19th, 2019 and July 2nd, 2019.

The following Fig. 4D is a plot of temperature, in °C, on the x-axis and depth, in m on the y-axis for Caesar Creek Lake for Year 1 (leftmost plot) and Year 2 (rightmost plot). A thermocline is observed around June 13th, 2019 in Year 1. Similarly, in Year 2, a thermocline is observed around June 5th, 2019. This implies that the thermal stratification in Caesar Creek Lake was similar between Year 1 and Year 2.

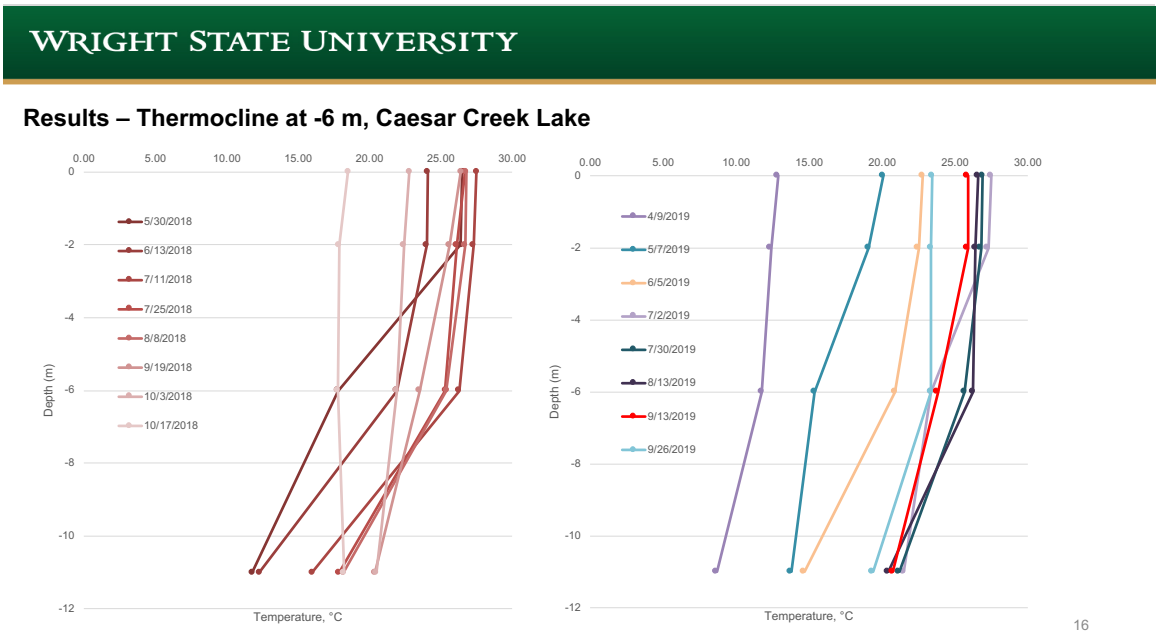
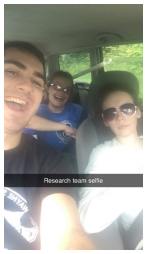
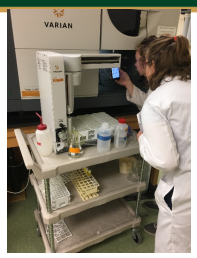


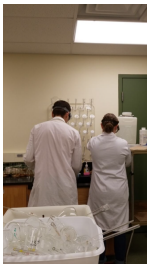
Fig. 4D - Plot of temperature, in °C, on the x-axis and depth, in m on the y-axis for Caesar Creek Lake for Year 1 (leftmost plot) and Year 2 (rightmost plot).).A thermocline is observed around June 13th, 2019 in Year 1. Similarly, in Year 2, a thermocline is observed around June 5th, 2019. This implies that the thermal stratification in Caesar Creek Lake was similar between Year 1 and Year 2.

Acknowledgements

- This work was funded by the Sture Fredrik Anilot Fund, the Clinton County Streamkeepers, and the WSU Department of Chemistry.
- Dr. Audrey E. McGowin
- The McGowin Team
- Lee Raska and Clara Leedy for their hard work and valuable contribution and 2d Lt Gary Foskuhl, Army, *USMA*, *'87*. Thanks to him, my courage never quits.
- Layla Foskuhl for her steadfast companionship and comfort.
- Dayton Public Schools, for their invaluable cultural and academic education







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More Pictures

45